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A Journal of Pulmonary Diseases

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1. Wilhelmj, C. M.; McDonough, J., and McCarthy, H. H.: Nutrition and Blood Pressure, Am. J. Digest. Dis. *20*:117 (May) 1953.
2. Mann, G. V., and Stare, F. J.: Nutritional Needs in Illness and Disease, J.A.M.A. *142*:409 (Feb. 11) 1950.
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4. Levine, V. E.: The Blood Pressure of the Eskimo, Federation Proc. *1*:121 (Mar. 16) 1942.

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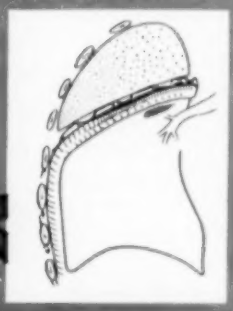

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A COMPARATIVE ANALYSIS OF THE POST-DISCHARGE EXPERIENCES OF TUBERCULOUS PATIENTS[1]

SOL L. WARREN[2]

(Received for publication May 5, 1953)

INTRODUCTION

As an organized program of public aid to the physically handicapped, vocational rehabilitation had its inception in this country some thirty-odd years ago. General acceptance of the program as a community responsibility is now rarely questioned. Increasingly large sums are being spent in its operation. Yet, despite the magnitude of the effort and the approbation it has received from professional and lay sources, its definitive values remain essentially unconfirmed.

To be sure, there have been studies which have attempted to evaluate such programs. Without underestimating their merits in advancing the field generally, however, the observation is inescapable that there is little in the literature which strictly satisfies the requirements of scientific inquiry and reporting. Compilations of closed case statistics (3, 13, 14),[3] inspirational accounts (4, 9, 15), administrative interpretations (5, 12, 16), personal testimony (1, 2, 10), and uncontrolled follow-up studies (7, 8, 11), while valuable in themselves, cannot be regarded as adequate substitutes for formal and rigorous analysis. More valid evidence of the merits of the program is needed, not only to convince sincere skeptics but to encourage ardent disciples.

If this criticism may be leveled at rehabilitation research in general, how much more cogent is it in the case of such a specialized area as tuberculosis rehabilitation? Kiefer (6), speaking before a conference of tuberculosis specialists in 1946, answered this question in these words, "To be perfectly frank about it, there is nothing in the literature . . . which can be used to prove that rehabilitation of the tuberculous is worth while." More recently, the National Tuberculosis Association (17) echoed Kiefer's remark by asking editorially, "Many rehabilitation workers suspect that the payoff is better, but how about some statistically valid inquiries to prove it?" It was a sharing of this recognition of the need for

[1] From the Municipal Sanatorium, Otisville, New York.

[2] Address: New York State Education Department, Division of Vocational Rehabilitation, New York, New York.

[3] The references cited in this paragraph are illustrative only.

systematic research in this field which prompted the evaluative study[4] described in the present report.

OBSERVATIONS

Objectives

The objectives of the study were:

> To discover whether any differences could be discerned between a group of persons who participated in a vocational rehabilitation program and a comparable group of persons who did not participate in such a program.
>
> To determine, if any such differences were found, their nature, extent, and significance to the individual, the rehabilitation agency, and the community.

In order to reduce to a workable number and variety the factors involved in setting up comparable groups, the inquiry was confined to a population of patients with a history of pulmonary tuberculosis who had been treated at and discharged from Municipal Sanatorium, a public institution maintained by the City of New York at Otisville, New York. The vocational rehabilitation program under evaluation was conducted by the New York City Office of the State Division of Vocational Rehabilitation.

Procedure

The most important characteristic of the study was its method of approach in setting up controlled conditions. To strengthen the validity of any conclusions which might be based on the findings, scrupulous attention was given to the three primary demands of scientific analysis in causal-comparative research. These include: verification of the comparability of experimental and control groups prior to the introduction of the experimental variable; maintenance of uniform conditions for the two groups during the conduct of the experiment; and the demonstration by valid statistical techniques of the subsequent presence or absence of significant differences between the groups.

Two hundred and fifty-seven patients discharged from Municipal Sanatorium during 1942 and 1943 were selected from the total discharge population for those years because they exhibited homogeneity with respect to a variety of pertinent factors. The patients were all first admissions to the hospital who had been discharged with medical consent as having arrested tuberculosis; they were all on four-hour activity tolerance, were considered to have favorable prognoses, and were in need of post-discharge rehabilitation assistance. The patients were all of equivalent economic status, having met the financial standards for admission as non-paying patients. They had all been processed through the rehabilitation program conducted at the sanatorium.

Following discharge, these patients separated into two groups as some availed themselves of the services provided by the state agency whereas others did not.

[4] This study was conducted in connection with a doctoral program at the School of Education, New York University, New York, N. Y.

TABLE 1

SERVICES RECEIVED FROM THE STATE AGENCY BY THE PARTICIPATING SUBJECTS

SERVICE RENDERED	NUMBER	PER CENT
Medical evaluation	79	100.0
Vocational evaluation	79	100.0
Counsel and guidance	79	100.0
Follow-up in employment	79	100.0
Vocational training	68	86.1
Maintenance allowances	48	60.8
Supplies and equipment	48	60.8
Psychometric evaluation	22	27.8
Job placement	12	15.2
Physical restoration	1	1.3

The nature and distribution of the services received by the participants is shown in table 1. It is obvious that some persons required the entire range of services in order to achieve vocational adjustment while others needed one combination or another.

In 1947 and 1948, precisely on the fifth anniversary of discharge from the sanatorium, both groups were followed up by personal interviews. Additional data were obtained by correspondence and other means from various pertinent sources, such as clinics, hospitals, physicians, social agencies, friends, relatives, and employers.

Of the 257 patients selected as subjects, 240 (93.4 per cent) were located and included in the study. The remaining 17 could not be found or refused to cooperate. Of the 240 subjects studied, 79 participated in the state agency's program while 161 failed to take part.[5]

The opposing groups were compared with respect to the pre- and post-discharge characteristics and experiences. Criteria of comparison included a variety of factors commonly associated with physical, emotional, social, economic, and vocational adjustment. Chi-square and t calculations were employed to discriminate between significant and chance variations. A 5 per cent level of confidence was established as the borderline of significance.

RESULTS

A pre-discharge comparison of the participating and non-participating groups disclosed their equivalence with respect to every criterion measured. Among these were such factors as age, sex, color, religion, place of birth, and citizenship status.

Socio-economic factors included marital status, number of dependents, source of income, public welfare experience, and frequency of contact with social agencies. The highest school grade completed and in-sanatorium activities were also equated, as were psychologic test results and personality characteristics.

[5] In order to simplify the subsequent discussion, these two groups are designated, respectively, *participants* and *non-participants*.

Under physical data, the comparison covered family tuberculosis history, interval between onset of symptoms and diagnosis and between diagnosis and admission to the sanatorium, length of stay and treatment received at the sanatorium, lung involvement, stage of advancement, clinical status, activity tolerance, and prognosis.

Included among the vocational factors were length of work history and number of occupations and jobs held. Moreover, a detailed comparison was made of the last pre-sanatorium job, including occupational classification, duration, how obtained, why relinquished, entrance and terminal earnings, wage increases and promotions, days and hours of work, vacation and sick-leave provisions, absence experience, and job satisfaction.

On the basis of the pre-discharge analysis outlined above, it was concluded that, at the point of discharge from the sanatorium, the participating and non-participating groups were comparable in every meaningful respect. The significance of this equivalence in providing a valid basis for conclusions bearing on the post-discharge experiences of the groups is obvious.

The post-discharge analysis involved a comparison of the groups with respect to all pertinent factors mentioned above in addition to many others. In fact, more than 70 separate items were included. The findings disclosed more favorable outcomes for the participating group in virtually every aspect investigated. Because of space limitations, only the more significant ones are presented here.

Physical Status

The participants exhibited considerably lower mortality and relapse rates than the non-participants. Whereas 18.0 per cent of the latter died of tuberculosis by the end of the fifth year, only 5.1 per cent of the former succumbed. Similarly, 61.5 per cent of the non-participants experienced a relapse at least once during the five-year interval, whereas only 25.6 per cent of the participants experienced recurrences.

Although the tuberculosis status of all subjects at the time of discharge was recorded as arrested, at the end of five years 81.0 per cent of the participants against 57.8 per cent of the non-participants were reported in this status. Moreover, 38.0 per cent of the former but only 18.0 per cent of the latter were completely discharged from medical supervision at the follow-up interview.

Similar one-sided comparisons were noted with regard to symptom reappearance, number of lungs involved, stage of disease advancement, prognosis, and activity tolerance. Although comparable in all of these respects at the time of discharge, at the follow-up interview those who had received rehabilitation assistance were uniformly better off than their non-participating counterparts.

Psychologic Status

The Bell Adjustment Inventory is designed to measure the degree of adjustment (or maladjustment) to one's home, health, social, emotional, and occupational environment. The sum of these adjustments furnishes the basis for an estimate of total personality adaptation. Measured by this criterion, 19.0 per

cent of the participants scored in the maladjusted range, whereas 44.5 per cent of the non-participants fell into this category. A breakdown of the scores shows that only in the health and occupational areas did significant disparities appear between the groups. In the remaining categories the differences, although uniformly in favor of the participants, were relatively small. Nevertheless, the extent of maladjustment exhibited by the non-participants in the health and occupational areas was sufficient to project many of the total scores into the unsatisfactory range, thus accounting for the large over-all disparity between the groups. It is interesting to note that physical and occupational adjustment represents the primary concern of the State Rehabilitation Agency.

Vocational Status

Compared at follow-up interviews, 84.8 per cent of the participants were employed in contrast to only 47.2 per cent of the non-participants who were so occupied. Moreover, the former spent 74.3 per cent of the sixty-month follow-up period in employment while the latter spent 57.0 per cent. The significance of this disparity is greater than is apparent in view of the forced unemployment imposed on the participants by reason of their acceptance of the rehabilitation program. The average time spent in the program was 11.3 months (18.8 per cent).

The non-participants spent 11.2 per cent of the follow-up period seeking employment while the participants used but 3.0 per cent of the time for this purpose.

The frequency of occupational and job change-over was studied for its bearing on vocational adjustment. On both scores, the participants manifested more favorable post-discharge histories. For example, while only 15.2 per cent of the participants changed occupations more than once during the five-year interval, 39.0 per cent of the non-participants did so. Likewise, only 19.0 per cent of the former but 40.9 per cent of the latter held four or more jobs during this period.

To be included in the study, each subject had to be in need of post-discharge rehabilitation assistance. In the majority of cases, the need was based on the necessity or desirability of re-directing the subjects' vocational goals. The nature and extent of re-direction which resulted is shown in table 2, which gives the occupational classification of the last pre- and terminal post-sanatorium jobs for the participants and non-participants.

It is evident that after discharge the non-participants reverted essentially to their pre-admission occupational status. The participants, on the other hand, exhibited a clear-cut trend out of the service and unskilled occupations into the professional, clerical, and skilled categories.

Earnings experience almost always receives a major emphasis in evaluative studies of rehabilitation, a prominence which is not wholly unwarranted. The interrelation between earning power and social, emotional, and economic factors makes it a matter of paramount concern to the individual. Its bearing on public income (from taxes) and expenditure (for welfare aid) makes it of equal concern to the community. The data on earnings are presented in table 3.

Although both groups earned approximately the same wages in their last pre-sanatorium jobs, the participants entered initial post-discharge jobs at decidedly

TABLE 2

PARTICIPANTS AND NON-PARTICIPANTS, BY OCCUPATIONAL CLASSIFICATION OF LAST PRE-SANATORIUM AND TERMINAL POST-SANATORIUM JOBS

OCCUPATIONAL CLASSIFICATION ACCORDING TO DICTIONARY OF OCCUPATIONAL TITLES (U. S. EMPLOYMENT SERVICE, 1939)	PER CENT OF SUBJECTS			
	Participants		Non-Participants	
	Last Pre-* (71)	Terminal Post- (79)	Last Pre- (146)	Terminal Post- (159)
Professional and managerial	4.2	13.9	5.5	5.0
Clerical and sales	21.1	34.2	19.2	20.5
Service	22.5	6.3	24.7	21.7
Agricultural	—	1.3	—	1.2
Skilled	11.3	26.6	10.3	10.6
Semi-skilled	22.5	12.7	21.9	21.1
Unskilled	18.3	5.1	18.5	18.6
No data	—	—	—	1.2
Total	100.0	100.0	100.0	100.0

* The figures in parentheses are the number of subjects.

TABLE 3

PARTICIPANTS AND NON-PARTICIPANTS BY AVERAGE WEEKLY EARNINGS IN PRE-SANATORIUM AND POST-SANATORIUM JOBS

	AVERAGE WEEKLY EARNINGS	
	Participants	Non-Participants
Last pre-sanatorium job		
Entrance earnings	$24.70 (68)*	$23.90 (137)
Terminal earnings	33.30 (70)	34.00 (144)
Initial post-sanatorium job		
Entrance earnings	28.40 (79)	37.50 (158)
Terminal earnings	36.90 (78)	40.60 (157)
Terminal post-sanatorium job		
Entrance earnings	40.90 (78)	37.30 (159)
Terminal earnings	52.50 (79)	44.20 (159)

* The figures in parentheses are the number of subjects.

lower wages than did the non-participants. By the time the initial job was relinquished, however, the differential between the groups was substantially reduced, an outcome attributable to the more favorable advancement experience and longer tenure enjoyed by the former. This trend continued. Thus, when the participants entered terminal employment, they were able to command slightly higher wages than the non-participants; and, finally, when terminal wages were recorded, the participants had forged ahead quite conclusively. Moreover, there was every indication that this gap would widen with the passage of time.

Without presenting details, it is recorded here that, when the jobs held by the participants and non-participants were analyzed with regard to other factors, almost invariably the former were found to be more advantageously situated.

This observation extended to such items as days and hours of work, total earnings, number of wage increases and promotions, job tenure, sick-leave provisions, employer awareness of the tuberculous background, and absenteeism.

In addition to studying those employment factors which lent themselves to objective measurement, the subjects were asked to evaluate their jobs subjectively in terms of over-all satisfaction. When the terminal post-sanatorium job was thus considered, 79.7 per cent of the participants but only 44.1 per cent of the non-participants expressed general satisfaction with their work. This is contrasted with 40.8 and 37.6 per cent of the groups, respectively, who signified approval of their last pre-sanatorium jobs. The best that may be said for the non-participants, then, is that they were not much worse off after discharge than before. On the other hand, the figures point to a definite improvement in the situation of the participants.

In order to compare job suitability, three specialists in the placement of the ex-tuberculous were asked to rate the terminal employment of both groups with respect to 25 separate criteria. Differences in favor of the participants were reported for 22 of the factors evaluated, 15 being of a statistically significant order. The 15 significant factors, in order of decreasing levels of significance were physical compatibility, general suitability of vocational choice, tenure, job satisfaction, existence of vocational problems, feeling of accomplishment, compatibility with aptitudes and interests, financial adequacy, relation to educational background, suitability of working hours, advancement opportunity, emotional security, compatibility with mental level, and sick-leave provisions. The seven remaining factors were: vacation provisions, social compatibility, remuneration in relation to prevailing wages, surroundings, relations with employer, compatibility with previous work background, and job location.

In the three instances which favored the non-participants, the differences were all nonsignificant. They were: recognition of employee accomplishment, degree of supervision, and relations with other employees.

On an over-all evaluation of job suitability, 78.5 per cent of the participants were judged satisfactorily employed as against 49.7 per cent of the non-participants. Conversely, 12.7 per cent of the former and 36.8 per cent of the latter were rated unsatisfactorily employed. It is significant that all three judges were in full agreement approximately 70 per cent of the time.

Socio-Economic Status

The participants evidenced a higher marriage and lower separation rate than the non-participants, a phenomenon probably related to the more favorable physical and vocational adjustment enjoyed by the former. Persons with relapsing tuberculosis requiring periodic hospitalization and those beset by adverse occupational and economic circumstances are less likely to assume the responsibilities of marriage than those who are more advantageously situated. By the same token, such adverse circumstances are more likely to predispose to broken marriages.

It was found that 82.3 per cent of the participants were financially independent

at the time their tuberculous condition was discovered. By the time of admission to the sanatorium, only 24.1 per cent were self-sustaining. Five years after discharge, the proportion reverted precisely to the original figure, 82.3 per cent. Truly, the definition of rehabilitation as the "restoration of the individual . . ." was demonstrated literally. By comparison, 84.5 per cent of the non-participants were self-supporting upon discovery of the disease, 25.5 per cent on admission to the sanatorium, but only 46.0 per cent at follow-up. Obviously, the latter fell far short of a full comeback.

Figures were computed to show the amounts expended for public assistance and the time spent "on relief" during the five-year interval. Including those subjects for whom no costs were involved, the average expenditure for relief was $204 per participant and $345 per non-participant. Using the same basis for computation, the participants, as a group, received public aid for 6.5 per cent of the follow-up period as against 13.3 per cent for the non-participants. In view of the fact that the former, by reason of their involvement in the rehabilitation program, were delayed in attaining financial independence and thereby obliged to seek public aid in considerable numbers during the period immediately following discharge, the over-all advantage displayed by them takes on added significance.

The average sums spent for hospitalization following discharge were $259 per participant and $737 per non-participant, the figures including those subjects who were not hospitalized at all. Thus, when the per capita cost of rehabilitating the participants ($225) is added to the costs of welfare and hospitalization for this group, the total comes to $688. For the non-participants, welfare and hospitalization costs alone amounted to $1,082 per case. It is noted that the cost of rehabilitation was nonrecurring and that welfare and hospitalization costs for the participants were on the decline at follow-up. For the non-participants, on the other hand, the trend was such that a much wider gap could be expected between the groups in the years subsequent to the survey.

The frequency of application to community agencies for help in resolving personal problems was studied. Although both groups were comparable in this respect in the pre-sanatorium period, a marked change occurred following discharge. Thus, for example, whereas 31.7 per cent of the non-participants sought the aid of more than two agencies, only 15.3 per cent of the participants felt obliged to do so. Apparently, with their problems defined and receiving attention from the rehabilitation agency, the participants did not have to spend as much time and effort as the non-participants in shopping around for help. Besides the advantages thus gained by the individual persons, the benefits to the community in terms of agency time and money saved are obvious.

DISCUSSION

On the basis of the findings disclosed by the present study, it may be concluded that participation in the vocational rehabilitation program produced consequences which were definitely significant to the persons who participated, to the rehabilitation agency which provided the services, and to the community as a whole.

In terms of individual experiences, participation in the program produced certain specific outcomes. In encouraging adequate convalescence and continued attention to medical advice, in discouraging premature resumption of employment, and in promoting the selection of suitable vocational objectives, it contributed significantly to the attainment and maintenance of normal health following discharge. In helping by supportive, inspirational, and tangible assistance to overcome the psychologic trauma generally associated with tuberculosis affliction, it contributed to the restoration of normal life patterns after discharge. In furnishing sound occupational guidance, adequate job preparation, placement assistance, and close supervision of employment, it contributed to satisfactory and successful vocational experience in occupations commensurate with mental levels, interests, aptitudes, aspirations, education, work background, and physical capacities. In advancing vocational development by providing useful skills in remunerative fields and by otherwise structuring the circumstances to produce high caliber performance which would merit employer recognition, it contributed to the enhancement of economic circumstances and the restoration of financial independence. In creating an advantageous physical, emotional, vocational, and economic climate, it contributed to the assumption and fulfillment of those community and family obligations which are essential to the social and personal adjustment of the individual.

As for the rehabilitation agency, the findings are significant in several ways. They confirm generally and establish specifically the values inherent in a program so long practiced yet so tenuously justified. They substantiate the economic soundness of the investment in the program. They provide the basis for continuing attempts to obtain future development and expansion of the program.

Finally, in terms of community involvement, the findings are significant because of the following considerations: They provide facts which should enlighten the public and management regarding the proficiency, stability, and vitality of ex-tuberculous workers properly placed. They furnish ammunition to all antituberculosis forces in their continuous compaign against this debilitating disease. They demonstrate the practical and tangible benefits to the community in terms of tax returns and purchasing power as well as in savings in welfare and hospitalization costs, all at the expense of a relatively small investment. They point the way to a happier citizenry through the promotion of economic self-sufficiency, physical and emotional well-being, social and vocational adjustment, and general personal contentment.

SUMMARY

A study was conducted to determine (*1*) whether any differences could be discerned between a group of ex-tuberculous sanatorium patients who, following discharge, participated in a state agency's vocational rehabilitation program and a comparable group which did not participate; and (*2*) if such differences were found, their nature, extent, and significance to the individual, the rehabilitation agency, and the community.

The findings disclosed that:

The subjects who participated in the rehabilitation program exhibited post-

discharge histories which were measurably different from those experienced by the non-participants, the differences being virtually always in favor of the former.

The differences between the participants and the non-participants were extensive, substantial, and influential. Their extensiveness was judged by their occurrence in each of the areas included within the definition of rehabilitation. Their substantial nature was attested to by statistical evidence of significance. Their influential nature was apparent from the critical consequences attending the presence or absence of particular attributes.

The differences between the participants and non-participants were attributed to the operation of the state agency's rehabilitation program, this being the only factor which functioned unilaterally during the post-discharge period.

The differences between the participants and non-participants were significant to the individual persons concerned, the rehabilitation agency, and the community.

SUMARIO

Análisis Comparativo de lo Sucedido a los Tuberculosos Después del Alta

Llevóse a cabo este estudio para determinar: (1) si podía descubrirse alguna diferencia entre un grupo de ex-enfermos de un sanatorio para tuberculosos que, después del alta, participaron en un plan de rehabilitación profesional a cargo de un organismo oficial y un grupo comparable que no participó en dicho plan; (2) si existían tales diferencias, cuáles eran su naturaleza, alcance e importancia para el individuo, el organismo de rehabilitación y la colectividad.

Los hallazgos revelaron que:

Los sujetos que participaron en el plan de rehabilitación acusaron, después del alta, una historia mensurablemente distinta de la de los que no participaron, redundando las diferencias virtualmente siempre en pro de los primeros.

Las diferencias entre los participantes y los no participantes fueron extensas, substanciales e influyentes. Su alcance fué apreciado a base de su existencia en cada una de las zonas comprendidas en la definición de rehabilitación. Su substantividad quedó comprobada por datos estadísticos de su importancia. Su influencia fué puesta de manifiesto por las consecuencias críticas que acompañaron la presencia o ausencia de ciertos atributos.

Las diferencias entre los participantes y los otros fueron atribuídas al efecto del plan de rehabilitación del organismo oficial, siendo ése el único factor que funcionó unilateralmente durante el período subsiguiente al alta.

Las diferencias entre los participantes y los no participantes revistieron importancia para las personas interesadas, el organismo de rehabilitación y la colectividad.

RESUME

Une analyse comparative des conditions de la post-cure de patients tuberculeux

Une étude a été entreprise pour déterminer: 1) si une différence quelconque pouvait être discernée entre un groupe de tuberculeux qui, après la fin de la cure en sanatorium, avait suivi le programme de réadaptation professionnelle et un groupe comparable n'ayant pas pris part au programme: 2) si des différences étaient observées, leur nature, leur étendue et leur portée à l'égard de l'individu, de l'organisme de réadaptation et de la collectivité.

Les résultats ont montré que:

L'histoire des sujets inclus dáns le programme de réadaptation différait appréciablement de celle des non participants, les différences étant toujours essentiellement en faveur des premiers.

Les différences entre les participants et les non participants étaient étendues, substan-

tielles et susceptibles d'influencer les résultats. Leur étendue était appréciée selon leur occurrence dans chacune des sphères comprises dans le domaine de la réadaptation. Leur qualité substantielle était attestée par des preuves statistiques. Leur influence se manifestait par les conséquences décisives entraînées par la présence ou le défaut d'attributs particuliers.

Les différences entre participants et non participants étaient attribuées à l'application du programme de réadaptation de l'organisation officielle, ceci représentant l'unique facteur fonctionnant unilatéralement durant la période de post-cure.

Les différences enregistrées entre participants et non participants avaient une signification statistique pour les individus intéressés, l'organisme de réadaptation et la collectivité.

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PRECLINICAL BRONCHOGENIC CARCINOMA[1, 2]

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INTRODUCTION

Bronchogenic carcinoma is increasing in incidence while resection rates remain under 50 per cent (1–4) of the cases diagnosed, and five-year survival rates continue to be less than 10 per cent (1, 3) of the resected cases. The question immediately arises whether the prognosis would improve if the characteristics of early lung cancer were more clearly delineated. Rigler (5), Overholt (6), and others state that roentgenographic changes suggestive of bronchogenic carcinoma precede the appearance of symptoms, but, on theoretical grounds, this cannot invariably be true. The chronology with respect to symptoms or roentgenographic changes depends on: site of origin, rate of tumor growth, degree of patient's perception of illness, index of roentgenographic suspicion, thoroughness of medical investigation, frequency of chest roentgenograms, and, possibly, other unrecognized factors. If roentgenographic changes precede symptoms, well-conducted surveys should provide better prognoses. This has not been the experience of the present writers, however, despite "over-reading" of survey films as "suspect neoplasm," efforts to hospitalize as emergencies patients whose films are so read, and routine persistent efforts to obtain prompt diagnostic studies. Accordingly, a more detailed study of the role of mass roentgenographic surveys in the detection of bronchogenic carcinoma has been conducted and forms the basis for the present report.

MATERIAL AND METHODS

In an effort to evaluate surveys as case-finding techniques for bronchogenic carcinoma, 77 consecutive proved cases of lung cancer, detected at two official Philadelphia chest roentgenographic units from 1947 through 1952, were studied. The cases were analyzed according to age, race, sex, presence or absence of symptoms, appearance of photofluorograms, resectability, tissue diagnosis, and five-year survival. In 22 instances, earlier photofluorograms were available for retrospective review.

Prevalence studies were limited to the 142,156 persons on whom appropriate records were available.

RESULTS

Careful follow-up of patients with any photofluorographic abnormality revealed bronchogenic carcinoma prevalence rates per 100,000 of 3 for females and

[1] From the Tuberculosis Control Section, Department of Public Health, Philadelphia, Pennsylvania.

[2] Presented in part before the Medical Session, as part of the session on *Nontuberculous Diseases of the Chest*, at the annual meeting of the National Tuberculosis Association, Los Angeles, California, on May 19, 1953.

34 for males. The prevalence rates were 30 for the group as a whole, 160 for all of those who were more than forty-five years old, and 260 for males more than forty-five years of age. These are conservatively calculated values as they exclude the cases of persons with clinical but not tissue diagnoses as well as those who cannot be located (table 1).

Presence of symptoms: Only 7 of the 77 persons were truly asymptomatic. Of the remaining 70, 43 considered themselves well but had symptoms which were elicited on careful questioning. The other 27 persons were sufficiently ill to have consulted physicians.

Thus, 50 of the carcinomas could be considered preclinical in the sense that, whether because of low threshold of perception, ignorance, or deliberate disregard of symptoms, the patients had made no effort to obtain medical advice. The only possible approach to the detection of these 50 carcinomas was through mass roentgenographic surveys (table 2).

Cough was the most frequent symptom. All symptoms were encountered in a higher proportion of the persons who ignored them than in the persons who had consulted physicians. It was startling to find that 11 persons who had expectorated blood had not sought medical care.

Amenability to resection: There was no striking difference in resection and survival rates between the "preclinical" symptomatic group and the 27 patients with manifest symptoms. However, all 7 of those without symptoms had lesions which were probably resectable at the time of the first abnormal photofluorograms.

TABLE 1

PREVALENCE OF PROVED BRONCHOGENIC CARCINOMA AMONG PERSONS REFERRED TO TWO OFFICIAL PHILADELPHIA ROENTGENOGRAPHIC UNITS* BY AGE, RACE, AND SEX

LESS THAN 45 YEARS OF AGE	TOTAL WITH ROENTGENOGRAPHIC EXAMINATIONS	TOTAL PROVED BRONCHOGENIC CARCINOMA	PER CENT
Male, white	26,916	1	0.004
Male, nonwhite	29,200	2	0.007
Female, white	30,495	1	0.003
Female, nonwhite	28,110	—	—
Total less than 45	114,721	4	0.003
MORE THAN 45 YEARS OF AGE			
Male, white	12,076	26	0.215
Male, nonwhite	4,501	17	0.378
Female, white	7,809	1	0.013
Female, nonwhite	3,049	—	—
Total more than 45	27,435	44	0.160

* Unit 1—Total 1947; foodhandlers only, 1949 through 1952.
Unit 3—Total 1949 through 1952.

TABLE 2

SYMPTOMS* IN ORDER OF FREQUENCY ACCORDING TO WHETHER OR NOT PHYSICIAN HAD BEEN CONSULTED AT TIME OF FIRST ABNORMAL PHOTOFLUOROGRAM

SYMPTOMS	NUMBER	PHYSICIAN CONSULTED†		SYMPTOMS IGNORED‡	
		Number	Per Cent of 27	Number	Per Cent of 43
Cough	35	10	37.0	25	58.0
Weight loss	24	7	26.0	17	40.0
Chest pain	17	4	15.0	13	30.0
Sputum	15	3	11.0	12	28.0
Hemoptysis	15	4	15.0	11	26.0
Dyspnea	12	4	15.0	8	19.0
Fatigue	10	2	7.0	8	19.0
Fever, chills, et cetera	10	5	19.0	5	12.0
Anorexia	3	—	—	3	7.0
Weakness	2	1	—	1	—
Hoarseness	2	2	—	—	—
Shoulder pain	2	1	—	1	—
Sense of heaviness	1	1	—	—	—
Dysphagia	1	—	—	1	—
Due to cerebral metastases	1	1	—	—	—

* Among the 70 symptomatic persons.
† 27 cases.
‡ 43 cases.

TABLE 3

RESECTION AND SURVIVAL ACCORDING TO CLINICAL STATUS AT TIME OF FIRST RECOGNIZED, ABNORMAL PHOTOFLUOROGRAM*

CLINICAL STATUS	TOTAL	NUMBER RESECTED	PER CENT RESECTED	NUMBER ALIVE	PER CENT ALIVE
"Preclinical"					
Asymptomatic	7	3	43.0	3	43.0
Symptomatic	43	11	26.0	6	14.0
Clinically manifest	27	8	30.0	5	19.0
Total	77	22	29.0	14	18.0

* Follow-up to March 1, 1953.

The 3 patients whose lungs were resected are still alive eleven to fifty-two months postoperatively. The inoperability of the other 4 asymptomatic persons may well have been due to refusal of hospitalization by the patient until metastasis had occurred, in one instance, and to errors in medical management or judgment in the other 3 instances (table 3).

Photofluorographic appearance: Since investigation of symptoms yielded so little of prognostic value, the photofluorographic readings were studied in the light of information on resection and survival. The 18 persons whose lesions were

so unimpressive as to have been missed or read only as "suspect tuberculosis" on the photofluorograms had a much better prognosis than the 59 with lesions definitely suggestive of tuberculosis or neoplasm (table 4).

When the photofluorographic appearance was suggestive of tuberculosis only, clinical study was concentrated on tuberculosis alone for too long a period. The following case illustrates this point.

TABLE 4

RESECTION AND SURVIVAL ACCORDING TO PHOTOFLUOROGRAPHIC FINDINGS ON FIRST ABNORMAL FILM*

PHOTOFLUOROGRAPHIC FINDINGS	NUMBER	NUMBER RESECTED	PER CENT RESECTED	NUMBER LIVING	PER CENT LIVING
False negative	11	5	45.0	5	45.0
Suspect	7	3	43.0	2	29.0
Tuberculosis only	25	5	20.0	3	12.0
Suspect neoplasm†	34	9	26.0	4	12.0
Total	77	22	29.0	14	18.0

* Follow-up to March 1, 1953.

† 0.3 per cent of all photofluorograms were so classified.

Case 1. H. B., a fifty-nine-year-old white bartender, had a photofluorogram on June 9, 1947, which was read as "moderately advanced active tuberculosis with infiltrations in the left upper lung field and at the right base." Suspicion of neoplasm was not mentioned. He was interviewed on June 20, 1947. No symptoms could be elicited. His private physician, to whom he had been referred, reported on June 30, 1947, that there were "no signs, symptoms nor sputum so no tuberculosis." On July 1, 1947, a chest clinic reported "reinfection tuberculosis." On the basis of one 14- by 17-inch roentgenogram, another physician reported "well healed tuberculosis." Four months later, on November 6, 1947, because of left chest pain of six weeks' duration and retinal detachment, the man was hospitalized. He died on November 28, 1947. Autopsy revealed adenocarcinoma of the left lung.

Carcinoma and tuberculosis: The finding of tubercle bacilli on a single smear is inadequate for diagnosis of tuberculosis. Even when repeated positive sputum examinations for *M. tuberculosis* confirm the radiologist's findings, the possibility of associated bronchogenic carcinoma must be kept in mind in men more than forty-five years of age. In this small series of 77 cases, 6 men, representing 8 per cent, had both active tuberculosis, with *M. tuberculosis* in the sputum, and carcinoma. This is approximately the same proportion as that reported by Fried (7), Martin and Beaudet (8), and Moll (9). All of the 6 men with both tuberculosis and carcinoma have died.

Symptoms without photofluorographic abnormalities: What of the group of persons with symptoms and normal-appearing photofluorograms? It can only be surmised what lies among this group by studying earlier photofluorograms available on the 22 persons in the series who later were proved to have cancer. On retrospective review, there were 11 whose previous photofluorograms were again interpreted to be normal. Only 3 of these had resectional surgery and all have

died. In 11 instances, erroneous interpretations of *normal* had been made on the last previous photofluorograms before those films were recognized as abnormal. Of these 11 patients whose films were first erroneously interpreted as normal, 4 underwent resection and are alive eight to fifty-two months following the procedure. A fifth man is alive but his lung was *not* resected.

Prognosis related to incorrect interpretation of initial photofluorograms: The better prognosis of the patients whose films were incorrectly read as normal compared with the outcome in the patients with truly normal films was surprising, and so was examined in detail. The two groups were comparable in age and tissue diagnosis. The median age for the patients with erroneously normal films was fifty-nine years as compared with fifty-eight years for those with truly normal films. There were 8 squamous and 3 undifferentiated carcinomas among the "erroneously normal film" group, whereas there were 9 squamous and 2 undifferentiated carcinomas among the "truly normal film" group. The two groups were quite comparable, therefore, although it is recognized that rate of tumor growth varies within the same histologic classifications. The difference between these two groups is undoubtedly related to the sites of origin of the tumors. Insufficient data are available to permit comparisons from this standpoint.

Unless super-voltage survey techniques become feasible, it is unlikely that three-quarters of bronchogenic carcinomas which arise in major bronchi can be detected roentgenographically until they have: (*1*) impaired bronchial drainage and thus given rise to distal suppuration; (*2*) partially blocked a bronchus, producing obstructive emphysema; (*3*) completely blocked a bronchus, producing atelectasis; or (*4*) grown so large as to present a visible mass. In only one-quarter of the cases can early detection of peripherally located cancers be anticipated because of their favorable situation, being surrounded by air-bearing parenchyma. Even then, such lesions when small may easily be overlooked on single photofluorograms if they lie behind bony structures or the heart.

The main difference in prognosis between the members of the two groups who had previous photofluorograms available for review may be due to the fact that the median interval for the erroneously interpreted "normal film" group was twelve months, while that for the patients with truly normal films was seventeen months.

Had provision been made for comparison of serial photofluorograms, it is unlikely that the films would have been erroneously interpreted as normal year after year as homogeneous shadows gradually increased in size. While it may be administratively difficult, investigation should be made of the magnitude of the yield when serial "normal" photofluorograms in men over forty-five years of age are compared.

In most instances, those who had available previous normal photofluorograms had had symptoms develop between the last normal film and the first abnormal one. This does nor necessarily imply that, if more frequent films had been obtained, roentgenographic changes might not have preceded symptoms. A sound evaluation of the relative roles of symptoms and roentgenographic changes will have to await the results of forward studies such as those of the Philadelphia Pulmonary Neoplasm Research Program.

TABLE 5

RESECTION AND SURVIVAL ACCORDING TO TISSUE DIAGNOSIS

TISSUE DIAGNOSIS	NUMBER	PER CENT OF TOTAL	NUMBER RESECTED	PER CENT RESECTED	NUMBER ALIVE	PER CENT ALIVE
Squamous	45	58.44	15	33.0	11	24.0
Adenocarcinoma	11	14.29	3	27.0	1	9.0
Undifferentiated	17	22.08	4	24.0	1	6.0
Definite tissue unspecified	4	5.19	—	—	1	25.0
Total	77	100.00	22	29.0	14	18.0

Resection and survival related to tissue diagnosis: In more than half the cases, the tissue diagnoses were squamous-cell carcinoma. Resection and survival rates were better for the patients with squamous-cell carcinomas than for those with adeno- or undifferentiated carcinomas (table 5).

Among the 63 persons in the series who died, the average length of survival from the time of the abnormal photofluorogram was 11 months for those in whom the lung was not resected compared with 16.1 months for those in whom the lung was resected.

DISCUSSION

Of 77 consecutive cases of bronchogenic carcinoma uncovered by survey, 50 could be considered preclinical in the sense that the patients had not consulted physicians; hence, survey techniques were the only possible means for detection of the tumor.

Only 7 of the 77 patients were truly asymptomatic. There was no difference in resection and survival rates between the 43 persons whose symptoms could be elicited only on careful questioning and the 27 whose illness had caused them to seek medical advice. In general, the prognosis appears no better in survey-discovered bronchogenic carcinoma than in lung cancer reported by thoracic surgeons. This seems due to the limitations of chest roentgenography, the low index of suspicion on the part of radiologists and clinicians, and to a lack of understanding on the part of patients so that they fail to obtain chest films often enough or fail to accept recommendations in regard to hospitalization and resection. Two-thirds of 22 patients who underwent resections are alive one to six years post-operatively as compared with 10 per cent of the 55 whose lungs were not resected.

While all of the 77 tumors in the present series were found because of roentgenographic changes, an examination of the records and films of the 22 persons for whom previous photofluorograms were available suggests that clinical study of symptomatic men more than 45 years of age with normal photofluorograms might yield better results. For the small group with peripherally located "silent" carcinomas, serial roentgenograms are the case-finding techniques of choice.

SUMMARY

The prevalence of proved bronchogenic carcinoma among 142,156 persons who were examined roentgenographically at two official Philadelphia survey units

was 30 per 100,000. Age and sex were important factors. The prevalence for those more than 45 years of age was fifty times that for those less than 45. The prevalence for males was more than ten times that for females. There was no difference in prevalence between whites and nonwhites.

Among 77 consecutive proved cases of survey-discovered bronchogenic carcinoma, the resection rate was 29 per cent; and, during a follow-up period of three months to six years, the survival rate was only 18 per cent.

Less than 10 per cent of the patients in the series were asymptomatic. In view of the poor prognosis, education is needed for both the public and the medical profession to emphasize that survey films do not constitute adequate study when symptoms are present. When symptoms are absent, chest roentgenograms are recommended every six months for men more than 45 years of age.

Among 22 persons with previous *normal* photofluorograms available, the films immediately preceding those read as abnormal were reviewed. Eleven were found to be definitely normal even on retrospective review, while 11 were found to have been erroneously interpreted as normal. The need for "over-reading" of survey films is obvious. Suspicion of neoplasm is indicated in men more than 45 years old when *any* film abnormality is present. Comparison of serial films on men more than 45 years old is wise even when readings have been designated normal.

All of the 11 patients whose photofluorograms were preceded by true normal films have died. The median interval between the normal photofluorograms and the first recognized abnormal photofluorogram was seventeen months, again suggesting the need for more frequent chest roentgenograms in older men.

There were 6 cases of concurrent active tuberculosis with sputum positive for *M. tuberculosis* and bronchogenic carcinoma. Even when the diagnosis of active pulmonary tuberculosis is unequivocal, the possibility of associated lung cancer should be borne in mind.

Evaluation of the carcinoma among symptomatic older persons with normal photofluorograms is indicated.

SUMARIO

Carcinoma Broncógeno Preclínico

Entre 142,156 personas examinadas roentgenográficamente en dos Unidades oficiales de Encuesta de la ciudad de Filadelfia, la frecuencia del carcinoma broncógeno confirmado alcanzó a 30 por 100,000. La edad y el sexo constituyeron factores importantes. La frecuencia en los sujetos de más de 45 años de edad fué cincuenta veces mayor que en los de menos de 45. En los varones representó más del décuplo que en las mujeres. No hubo diferencia en los coeficientes entre las personas blancas y las de otras razas.

Entre 77 casos consecutivos comprobados de carcinoma broncógeno descubierto en las encuestas, el coeficiente de resección fué de 29 por ciento; y, durante un período de observación subsiguiente que duró de tres meses a seis años, el coeficiente de sobrevivencia no pasó de 18 por ciento.

Menos de la décima parte de los enfermos eran asintomáticos. Visto el pronóstico desfavorable, se necesita educar tanto al público como a la profesión médica a que comprendan bien que las radiografías de las encuestas no constituyen un estudio adecuado cuando existen síntomas. Si no los hay, recomiéndanse radiografías torácicas cada seis meses para los varones de más de 45 años de edad.

En 22 personas para las que había a mano previas fotorroentgenografías negativas, se estudiaron las que precedían inmediatamente a las consideradas como anormales. Once resultaron ser negativas aun repasadas retrospectivamente, en tanto que en 11 se descubrió que habían sido interpretadas erróneamente como negativas. Resulta manifiesta la necesidad de repasar cuidadosamente las radiografías tomadas en encuestas. Queda indicada una sospecha de neoplasia en varones de más de 45 años siempre que exista la menor anormalidad. Aun siendo negativas las lecturas, es prudente comparar radiografías seriadas en varones de dicha edad.

De los 11 cuyas fotorradiografías fueron precedidas de radiografías verdaderamente negativas, todos han muerto. El tiempo transcurrido entre las fotorroentgenografías negativas y la primera fotorroentgenografía reconocida como anormal promedió diecisiete meses, sugiriendo de nuevo la necesidad de obtener radiografías torácicas con más frecuencia en los varones de cierta edad.

Hubo 6 casos de coexistencia de tuberculosis activa con esputo positivo para el *M. tuberculosis* y carcinoma broncógeno. Aun siendo indudable el diagnóstico de tuberculosis pulmonar activa, hay que tener presente la posibilidad de cáncer pulmonar concomitante.

En las personas asintomáticas de cierta edad con fotorroentgenografías "negativas," está indicada la consideración del carcinoma.

RESUME

Cancer bronchogène préclinique

La fréquence du cancer bronchogène confirmé, parmi les 142.156 individus qui furent examinés radiologiquement dans deux unités officielles de l'Enquête de Philadelphie, atteignait 30 pour 100.000. L'âge et le sexe constituaient des facteurs importants. La fréquence, chez les sujets au-dessus de 45 ans, était cinquante fois plus élevée que celle enregistrée chez ceux au-dessous de 45 ans. La fréquence était dix fois plus élevée chez l'homme que chez la femme. Il n'y avait pas de différence de fréquence entre la race blanche et la noire.

Parmi une série de 77 cas de cancer bronchogène confirmé, qui ont été révélés par l'enquête, le taux des résections a été de 29 pour 100; le taux de survie n'a été que de 18 pour 100 pendant une période d'observation de trois mois à six ans.

Un dizième seulement des cas de cette série était asymptomatique. En raison du pronostic défavorable, il y a lieu d'instruire à la fois le public et les médecins pour bien montrer que les radiographies des programmes d'enquête ne constituent pas une étude adéquate quand les symptômes existent. En l'absence de symptômes, il est conseillé de faire des radiogrammes du thorax tous les six mois chez les hommes de plus de 45 ans.

Parmi 22 individus dont les radiogrammes antérieurs étaient négatifs, on a procédé à un nouvel examen des films précédant immédiatement les clichés considérés anormaux. Dans onze cas l'interprétation négative a été confirmée par l'examen rétrospectif; dans onze cas l'interprétation négative a été trouvée erronée. La nécessité d'un examen minutieux des clichés effectués au cours des enquêtes est évidente. Toute anomalie apparaissant dans un cliché chez un homme ayant dépassé 45 ans doit faire penser au néoplasme. La comparaison des films en série chez les sujets de plus de 45 ans est une mesure de prudence, même lorsque l'interprétation des clichés a été négative.

Les 11 sujets, dont les radiogrammes positifs avaient été précédés par des clichés absolument négatifs, sont morts. L'intervalle moyen entre les radiogrammes négatifs et les premiers clichés reconnus anormaux était de dix-sept mois, ce qui indique encore le besoin de radiogrammes plus fréquents chez les individus ayant dépassé 45 ans.

Il y avait 6 cas où la tuberculose en activité avec crachats positifs pour le *M. tuberculosis* était associée au cancer bronchogène. Même quand le diagnostic de tuberculose pulmonaire en activité est certain, il y a lieu de penser qu'il peut exister un cancer du poumon.

En présence de symptômes pathologiques chez des sujets d'âge mûr dont les radiogrammes sont "négatifs" la recherche du cancer est indiquée.

Acknowledgment

The authors are indebted to Minerva Waissman, R. N., for her tireless efforts in following patients; to Mr. William J. Carnahan, Research Secretary of the Philadelphia Tuberculosis and Health Association, for invaluable statistical help; and to Mrs. Alberta Connor for the preparation of the manuscript.

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AN EVALUATION OF CHEMOTHERAPEUTIC AGENTS IN THE CONTROL OF EXPERIMENTAL INFECTIONS DUE TO MYCOBACTERIUM LEPRAE MURIUM[1]

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INTRODUCTION

The ultimate decision concerning the efficacy of any antimicrobial agent in the treatment of leprosy will be reached only through extensive clinical study. Such clinical investigation is necessarily costly and time consuming, however, and must be limited to an evaluation of carefully selected compounds. Such studies are complicated, moreover, by the lack of established criteria upon which to base an evaluation of drug effects in a disease such as human leprosy. It has been suggested that precise information concerning the activity of possibly useful compounds in an experimental disease caused by microorganisms possessing the intracellular growth characteristics of human leprosy bacilli might assist in the recognition and development of compounds worthy of clinical trial. Due to the slow development of murine leprosy, however, the classical methods of study of this experimental disease to date have suffered from many of the same difficulties encountered in clinical work.

In the present investigation, an attempt has been made to ascertain if short-term chemotherapeutic trials in mice, similar to those now widely used in the evaluation of antituberculous agents, are adaptable to murine leprosy. In the absence of distinctive gross pathology and of the ability to make microbial counts by the conventional pour-plate technique, evaluation has been based on microscopic estimates of the numbers of stainable bacilli in the organs of infected animals at various time periods.

Credit for development of the technique described herein is due in large part to one of the four writers (J. H. H.) whose experience with the murine leprosy bacillus over a period of many years made it possible for him to outline a feasible procedure and to postulate in advance the nature of the results which could be obtained with it. The technique is based in part also upon studies reported by Grunberg and Schnitzer (1) and on techniques used previously for evaluation of antituberculous compounds (2–4).

MATERIALS AND METHODS

All experiments were conducted with the Hawaiian strain of the murine leprosy bacillus which was maintained in viable form by serial passage at six-month intervals in the Wiersing strain of white rats.

[1] From the Research Division, Chas. Pfizer & Co., Inc., Brooklyn, New York, and Leonard Wood Memorial Bacteriological Laboratory, Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts.

Mice of the Dba strain[3] (males weighing 18 to 22 gm.) were used throughout all chemotherapeutic studies. The animals were infected intraperitoneally with 0.4 ml. of a 2 per cent tissue homogenate (T_2) of *Mycobacterium leprae murium* from rat testis.[3] In each instance the testicular suspension was obtained, by the method described below for mouse spleen homogenates, from a rat which had been infected intratesticularly with 0.2 ml. of rat testis suspension (T_2) six months previously.

In chemotherapeutic studies, a minimum of 10, and frequently 20, mice were used for each dosage of each drug tested. Unless otherwise specified, treatment was started twelve hours after infection and was continued for a period of twenty-nine to forty-two days, at which time the animals were sacrificed.

Spleens: At autopsy the spleens were removed aseptically. Those from each set of animals were pooled, weighed, and treated in the following manner:

Preparation of tissue homogenate: Small pieces of the previously weighed tissue[4] were placed between the blades of a Waring Blendor which had been chilled overnight at −80°C. The amount of sterile distilled water necessary to yield a 10 or 20 per cent tissue suspension (T_{10} or T_{20}) was calculated and a portion of this was pipetted into the blender. The tissue was blended for two periods of thirty seconds each, with an interim period of thirty seconds. The blend was then transferred to a series of Porter Mills,[5] care being taken to rinse the blender well with a small quantity of sterile distilled water which was then added to the blend. The blended suspension was then homogenized for four minutes, the concentration of tissue brought to T_{10} or T_{20}, as desired, and the homogenate transferred to screw-cap tubes.

Population counts: The homogenate was diluted in 1 per cent formalin-milk,[6] as required, and standard 2 mm. loopfuls were spread uniformly over a series of four standardized 8 mm. circles on a glass slide. Smears were dried over a water-bath at 100°C., allowed to cool, and then covered with a solution of 0.5 per cent gelatin in 0.5 per cent phenol. Smears were again dried by heating over a water bath at 100°C. Slides were placed in formalin fumes for three to five minutes, heated to drive off the formalin fumes, and finally stained by the Ziehl-Nielsen technique.

Microscopic counts of the numbers of acid-fast bacilli present in each of four of the 8 mm. circular smears, using fields across the diameter of the smear at 1 mm. distances, were made on each tissue homogenate, i.e., the slide was moved 1 mm. at a time, as indicated by a substage micrometer, to allow examination of 9 fields per smear. Only the organisms in the portion of each field which fell within the area defined by a 5 mm. counting square[7]

[3] Obtained from Rockland Farms, New City, New York.

[3] Estimated number of bacilli in challenge dose: approximately 1.5 to 2.0 billion cells.

[4] Prior to blending, testicular tissue to be used for infection of other rats and/or mice was tested with tetrazolium. One per cent of tetrazolium violet in water was used as stock solution. A 0.2 per cent solution in 0.15 M phosphate buffer (pH 7.5) was prepared from the stock solution as needed. A small piece of tissue was placed in 0.5 ml. of the 0.2 per cent solution and allowed to stand for fifteen minutes. Tissue suitable for use became deep red in color. Tissues showing a color reaction of less than two plus to three plus were discarded as unsuitable for use.

[5] Manufactured by Macalaster & Bicknell, Inc., Cambridge, Massachusetts. Used with Motor No. 5230, 115 volts, type B, 50–60 cycles: H. M. Welch Scientific Co.

[6] Prepared as follows: Dissolve 10 gm. of Difco purple milk in 90 ml. of 1/150 M Na_2HPO_4; add 30 drops of IN—NaOH; then add to the milk slowly a mixture of 5 ml. of 10 per cent potassium alum and 1 ml. of formalin, while shaking to disperse the precipitate. Bring volume to 100 ml. by addition of 10 per cent purple milk. Centrifuge for five minutes at approximately 500 revolutions per minute. Draw off supernatant and discard sediment. Dilute to 1 per cent milk by addition of 0.5 per cent formalin. The optimum dilution of homogenate depends upon the number of bacilli present in the tissue and was determined for each tissue homogenate. Ten bacteria per field were considered optimum for counting.

[7] American Optical Co. eyepiece No. 422; net reticule, 21.15 mm. diameter, 5 mm. square ruled into 0.5 mm. squares.

were included in the counts (5). In general the counts on the 9 fields in any given smear were approximately 1, 2, 3, 3.3, 3.5, 3.2, 3, 2, and 1 times the number observed in the first field.

The population count on any tissue homogenate was calculated according to the formula:

$$T_{20} \text{ population count} = \frac{\text{total number of bacilli counted}}{\text{total number of fields counted}} \times \text{dilution in formalin milk} \times \frac{20}{\text{per cent tissue}}$$

RESULTS

Evaluation of the Method

The first group of experiments conducted by this procedure were designed to test the validity of the technique.

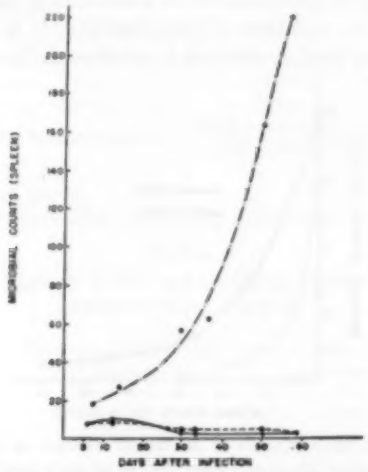

FIG. 1. Populations of *M. leprae murium* in mice: microbial counts of spleen suspensions (per cent tissue: T_{20})

○·—·—·○ Viable suspension
○————○ Heat-treated suspension (30 minutes—60°C.)
●- - - -● Heat-treated suspension (5 minutes—100°C.)

Multiplication of M. leprae murium in mice as evidenced by microbial counts on spleen suspensions: Preliminary experiments were conducted to determine the rate at which *M. leprae murium* increased in number in spleen suspensions of mice infected according to the method described above. Counts were made for comparison at 7, 14, 29, 36, 50, and 57 days after injection, on spleen suspensions of animals injected intraperitoneally with suspensions of viable bacilli and with organisms previously heated at 60°C. for thirty minutes and at 100°C. for five minutes. The results obtained in this experiment are illustrated in figure 1. Following injection of suspensions of viable bacilli, organisms were detectable in spleen suspensions within 7 days. The actual bacillary counts increased steadily during the first 30 days after infection and rose sharply thereafter. There was no increase over a 60-day observation period in the number of acid-fast organisms detected in spleen suspensions of animals injected with heat-treated bacilli, the

number of organisms remaining persistently low. It is of interest, however, that the spleen suspensions from animals injected with heated bacilli were never totally free of stainable acid-fast bacilli. The range between the population curves obtained with viable and with heat-treated bacilli in this experiment was large and it seemed probable, therefore, that drug evaluation would be possible between the thirtieth and sixtieth days.

Effect of heat upon viability of M. leprae murium: Population curves on spleen suspensions from mice injected intraperitoneally with heat-treated suspensions of *M. leprae murium* indicated a gradual decrease in the numbers of stainable cells as the length of the heating period was increased. In all instances, however, small numbers of stainable bacilli were apparent in the tissue suspensions. The resultant curves (figure 2) perhaps serve as a measure of the ability to kill *Mycobacterium leprae murium*, and thus of their viability. It is obvious that, if heat-treated suspensions are used as controls in studies of this nature, the period of

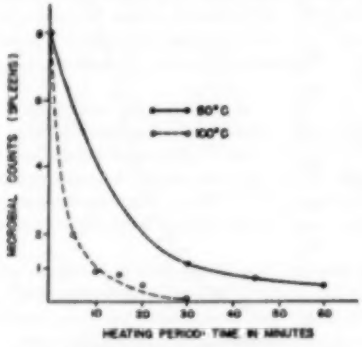

FIG. 2. Effect of heat upon inocula of *M. leprae murium* as evidenced by population curves *in vivo*. Spleen counts (per cent tissue: T_{25}) at 24 days post infection.

heating must be standardized. Comparable spleen homogenates from uninfected mice invariably showed no stainable acid-fast bacilli.

Reproducibility of population counts within the same laboratory: In a single experiment, two series of animals were infected simultaneously and treated identically throughout the course of a 30- to 31- day period. The animals were then sacrificed and population counts made independently on each of the two series of animals. The results shown in table 1 indicate that departures from the mean as great as plus-minus 20 per cent occur rarely when the bacterial populations in comparable tissue suspensions are counted by the same operator working under constant conditions.

Reproducibility of results based on population counts made in different laboratories: In a subsequent experiment, two series of animals were again infected simultaneously and treated identically throughout the course of a 30- to 31-day period. At the end of this period, the animals were sacrificed and, on the day of sacrifice, half were forwarded to an independent laboratory (*B*) where spleen homogenates were prepared and population counts were made simultaneously with, but independent of, the original laboratory (*A*).

TABLE 1

REPRODUCIBILITY OF POPULATION COUNTS MADE IN SAME LABORATORY (*A*)

(Mice)

DRUG	ORAL DOSAGE	SERIES NO. 1		SERIES NO. 2	
	Mg. Per Mouse Per Dose	Spleens		Spleens	
		No./Wt. (Gm.)	Counts*	No./Wt. (Gm.)	Counts*
Promin	50 mg. daily	9/2.7	22.9	8/2.3	16.6
Iproniazid	2.5 mg. daily	8/1.9	1.0	8/1.6	1.2
Promin	50 mg. twice weekly	8/2.0	16.2	9/2.1	16.1
Amithiozone	50 mg. twice weekly	8/2.2	13.1	7/2.0	10.0
Iproniazid	2.5 mg. twice weekly	10/2.8	5.4	10/2.3	5.7
Isoniazid	2.5 mg. twice weekly	10/2.0	3.9	10/2.2	3.9
Controls					
Infected with viable bacilli		9/2.0	20.4		
Injected with heated bacilli: 100°—5 minutes		10/2.3	1.1		
Injected with heated bacilli: 60°—30 minutes		9/1.9	1.9	9/2.0	1.6
Uninfected		10/1.4	0		

* Population counts on T_{20} suspensions = $\frac{\text{total number bacilli counted}}{\text{total number fields counted}} \times$ dilution in formalin milk $\times \frac{20}{\text{per cent tissue}}$.

All counts made at 31 days after infection by Laboratory *A*.

TABLE 2

REPRODUCIBILITY OF RESULTS BASED ON POPULATION COUNTS MADE IN DIFFERENT LABORATORIES (*A* AND *B*)

(Mice)

EXPERIMENT NUMBER	DRUG	ORAL DOSAGE	LABORATORY A*		LABORATORY B†
		(Mg. Per Mouse Per Day)	Spleens		Spleens
			No./Wt. (Gm.)	Counts	Average Bacilli Per Field
1	Amithiozone	50	5/1.8	11.0	64
	Isoniazid	2.5	9/2.4	1.0	6
	Controls				
	Infected with viable bacilli		9/2.0	20.4	59
	Injected with heated bacilli: (100°C—5 minutes)		10/2.3	1.1	7.5
	Uninfected		10/1.4	0	0
2	Controls				
	Infected with viable bacilli		5/2.4	40.3	186
	Injected with heated bacilli: (100°C—5 minutes)		10/1.3	3.2	7
	Injected with heated bacilli: (60°C—30 minutes)		10/1.9	3.1	11
	Uninfected		10/1.9	0	0

* Population counts: See legend for table 1. Counts made at 30 to 31 days after infection by Laboratory *A*.

† Population counts: See legend for table 1. Counts made by Laboratory *B* on mice infected and treated by Laboratory *A*. Counts made at 30 to 31 days after infection, on tissue suspensions made with 2 ml. of distilled water per spleen.

The results obtained by the two laboratories on comparable sets of animals are illustrated in table 2. The actual counts obtained by the two laboratories differed significantly. This may be accounted for readily by the fact that laboratory *B* made individual counts on 3 to 4 animals (unpooled) per set, using spleen suspensions prepared by the addition of 2 ml. of water to each spleen, irrespective of weight. Laboratory *A*, in contrast, used a 20 per cent (T_{20}) pooled spleen suspension throughout.

The differences in bacillary counts were minimized if the data of laboratory *A* were calculated for the dilution system used in laboratory *B* and the ratios between control and drug-treated animals were then determined for each set of data.

It is of interest that the relative effectiveness of isoniazid, as compared with amithiozone, was the same in both laboratories.

Reproducibility of population counts over a two-year period: The reproducibility of results from experiment to experiment, conducted in a single laboratory over a two-year period, is illustrated in figure 3. Population counts on control animals from 14 different experiments are shown. All counts were made by a single operator. The range between the counts on different sets of mice at the same period was narrow. Despite this, it is recommended that counts on drug-treated animals be compared only with counts on control animals infected and tested simultaneously with the treated animals. Use of average or composite curves,

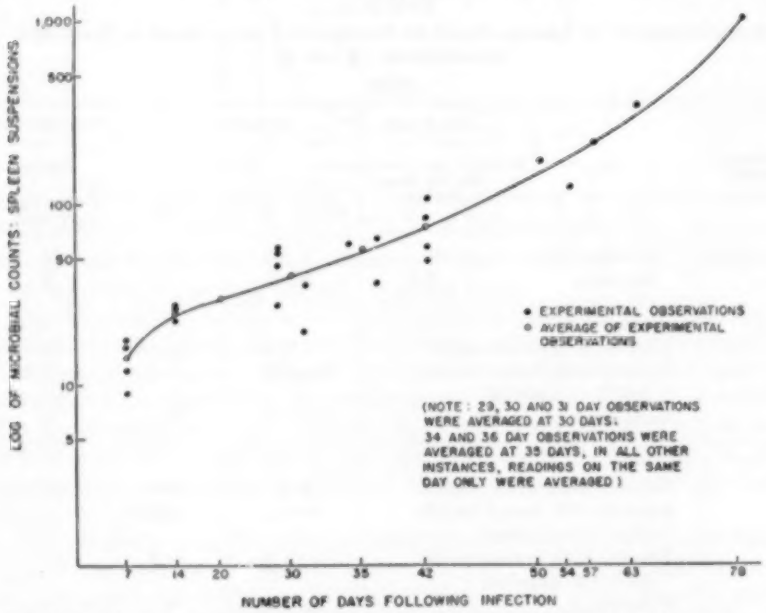

FIG. 3. Population counts on spleen suspensions from untreated control mice: composite data from 14 experiments. (Tissue suspensions: per cent tissue: T_{20})

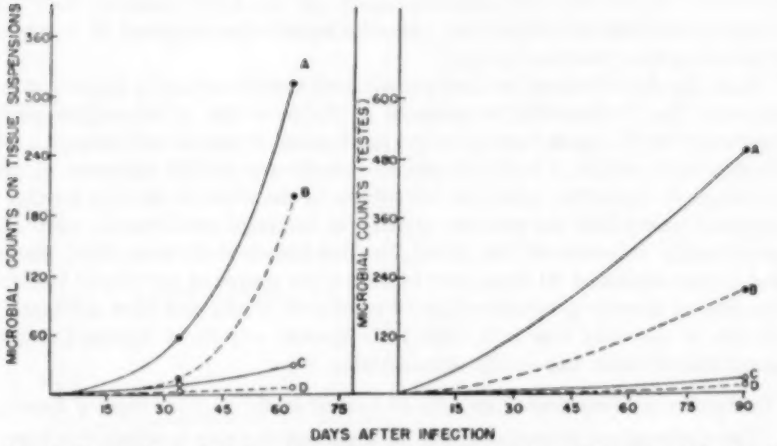

FIG. 4. Populations of *M. leprae murium* in tissue suspensions of drug-treated animals Subcutaneous therapy.

Mice: spleen counts: per cent tissue: T_{20}
Curve *A*—Saline 0.1 ml. per mouse b.i.d.
Curve *B*—Promin 10 mg. per mouse q.d.
Curve *C*—Isoniazid 0.5 mg. per mouse b.i.d.
Curve *D*—Isoniazid 1.25 mg. per mouse b.i.d.

Rats: testicular counts: per cent tissue: T_{20}
Curve *A*—Saline 0.1 ml. per rat b.i.d.
Curve *B*—Isoniazid 1.25 mg. per kg. b.i.d.
Curve *C*—Isoniazid 2.5 mg. per kg. b.i.d.
Curve *D*—Isoniazid 5 mg. per kg. b.i.d.

such as the one shown in figure 3, is not recommended due to possible variation in the testicular suspensions used for infection of the mice.

The effect of various dosages of isoniazid upon population counts in mice and rats: Evidence has been presented above to show the range of population counts in spleen suspensions of mice injected intraperitoneally with viable and with heat-treated suspensions of *Mycobacterium leprae murium*. Evidence has been presented also to establish the reproducibility of counts made under controlled conditions.

Preliminary observations on the action of Promin and of isoniazid on the population counts in spleen suspensions of mice infected with *Mycobacterium leprae murium* were conducted to evaluate the use of this technique for chemotherapeutic studies and to evaluate the extent to which variation in dosage of an effective drug would modify the population count.

Mice infected intraperitoneally with *M. leprae murium* were treated by the subcutaneous route, once daily, with varying dosages of isoniazid and with Promin for periods of 34 and 63 days, respectively. At the end of the treatment period, animals were sacrificed and the number of stainable acid-fast bacilli was determined in spleen homogenates. For comparison, a similar experiment was

conducted in rats infected intratesticularly. In the latter instance, microbial counts were made at 90 days on testicular suspensions prepared in a manner identical to that described above.

From the data obtained in these experiments and illustrated in figure 4, it was apparent that a quantitative measure of the activities of chemotherapeutic agents against *M. leprae murium* might be obtained in mice as well as in rats. The bacteriostatic action of isoniazid and of Promin was readily apparent in mice examined 34 days after infection. Differences in the effect of the two dosages of isoniazid tested and the relative activity of isoniazid and Promin were only questionably apparent at this period. In mice examined 63 days after infection and in rats examined 90 days after infection, the degree of activity of isoniazid was almost directly proportional, until maximum effects had been achieved, to the size of the daily dose and, indeed, the greater activity of isoniazid as compared with Promin was readily demonstrable.

Evaluation of Compounds Currently of Interest in the Chemotherapy of Leprosy

The observations presented above have defined the way in which this type of experimental infection develops in mice, and have indicated furthermore that the method described should be adequate for evaluation of chemotherapeutic agents active against *Mycobacterium leprae murium*. Thus, to establish a baseline for future comparison and to test the method further, it seemed advisable to evaluate the compounds of current interest in the chemotherapy of leprosy, using various dosage regimens. In addition, certain other readily available antimicrobial agents were included in the study.

Chemotherapeutic action of drugs administered daily to animals started on therapy within 12 hours after infection: Using the technique discussed above, mice infected with *Mycobacterium leprae murium* were treated daily with various dosages of iproniazid, isoniazid, Promin, amithiozone, 4,4′-diaminodiphenyl sulfone, streptomycin, streptomycin in combination with isoniazid, oxytetracycline (Terramycin[5]), and actithiazic acid (6). Treatment was started by the subcutaneous route 12 hours after infection and continued for periods ranging from 30 to 42 days, at which time the animals were sacrificed and microbial counts on spleen suspensions were made.

In the dosages used, oxytetracycline (2.5 and 5.0 mg. per day) and actithiazic acid (10 and 20 mg. per day) proved ineffective. The results obtained with the remaining compounds are illustrated in figure 5, in which activity is expressed in terms of the ratio of population counts on control animals to those on treated animals. For the purpose of evaluation, this ratio has been compared to the *maximum anticipated response* which was accepted as being equivalent to the ratio between the population count on spleen suspensions from control animals and that on spleen suspensions from animals injected with heat-treated bacilli (100°C. for 5 minutes).

Based on the data obtained in these experiments, it is apparent that isoniazid and streptomycin, alone and in combination, are far more effective than the widely used antilepromatous agents, Promin and 4,4′-diaminodiphenyl sulfone.

[5] Proprietary name of Chas. Pfizer & Co., Inc., Brooklyn, New York.

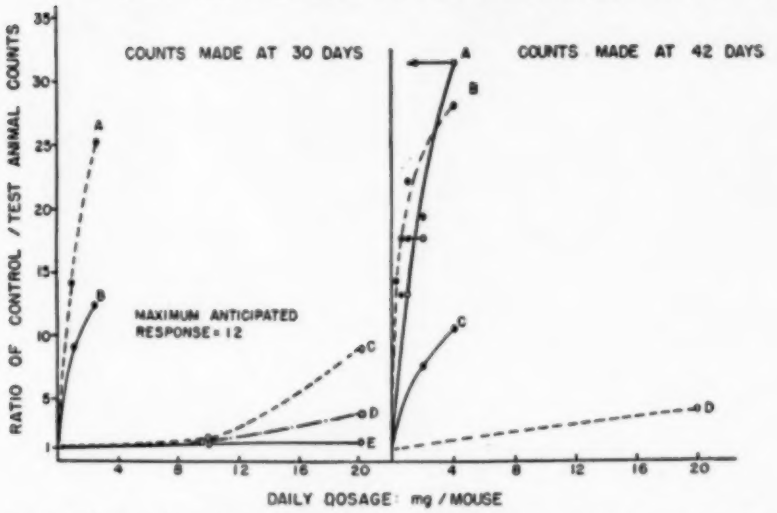

FIG. 5. Effect of drug therapy in infections due to *M. leprae murium* as evidenced by population counts on mouse spleen suspensions (per cent tissue: T_{20}). Subcutaneous therapy. Efficiency of drugs is expressed in terms of the ratio of population counts on control animals to those on treated animals.

Left:

Curve *A*—Isoniazid
Curve *B*—Iproniazid
Curve *C*—Amithiozone
Curve *D*—4,4′-diaminodiphenyl sulfone
Curve *E*—Promin

Right:

Curve *A*—Streptomycin plus isoniazid (plotted on basis of streptomycin dosage; 0.25 mg. isoniazid with each 1.0 gm. streptomycin: arrows indicate pull of curve to left to plot on basis of isoniazid content).
Curve *B*—Isoniazid
Curve *C*—Streptomycin
Curve *D*—Promin

Chemotherapeutic action of drugs administered daily to animals started on therapy 8 to 21 days after infection: Subsequent experiments were conducted to evaluate the ability of isoniazid to control the infection when start of therapy was delayed until microbial counts on spleen suspensions of sacrificed control animals showed significant numbers of acid-fast bacilli. Counts on untreated control animals were made the day before the start of therapy and simultaneously with all counts on drug-treated animals. Therapy was started in two series of animals on the eighth day, in four series on the fifteenth day, and in two series on the twenty-first day following infection. Treatment was continued until the day on which animals were sacrificed for evaluation, e.g., 21 or 42 days of therapy. The results are illustrated in figure 6.

From the results obtained in these experiments, it is apparent that, under the conditions used, isoniazid in dosages of 1 and 2 mg. per mouse per day was

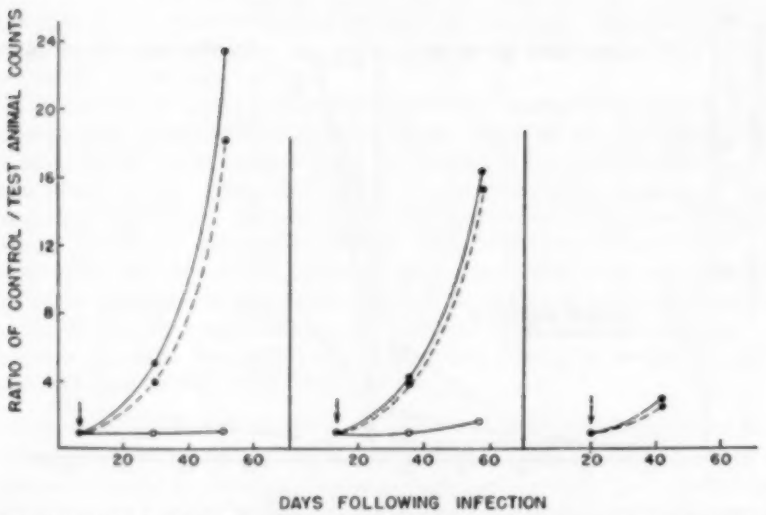

FIG. 6. Effect of delayed therapy with isoniazid and Promin upon populations of *M. leprae murium* in mice, as evidenced by spleen counts (per cent tissue: T_{20}). Therapy started on eighth day (left curve), fifteenth day (center curve), and twenty-first day (right curve) of infection.

●——● Isoniazid 2 mg. per day; ●- - -● Isoniazid 1 mg. per day; ○——○ Promin 10 mg. per day. Efficiency of drugs is expressed in terms of the ratio of population counts on control animals to those on treated animals.

capable of preventing further increase in the numbers of acid-fast bacilli in spleen suspensions. In all isoniazid-treated animals, there was indeed a gradual decrease in the number of bacilli present to approximately one-half of the number present at the start of therapy. The effect of Promin, in the dosages used, was negligible under the conditions of these experiments, the number of bacilli in spleen suspensions approximating at each time period the number in untreated control animals.

Experiments conducted with carbomycin (2.5, 5, and 10 mg. per mouse per day subcutaneously and 5, 10, and 20 mg. per mouse per day orally) as well as oxytetracycline (5, 10, and 20 mg. per mouse per day orally) indicated that these agents were ineffective when administered to mice beginning on the eighth and continuing to the twenty-ninth day after infection.

Chemotherapeutic action of drugs administered intermittently: In figure 7*A* the results obtained in a comparative study of daily and intermittent therapy of mice infected intraperitoneally with *Mycobacterium leprae murium* are illustrated. Promin, amithiozone, iproniazid, and isoniazid were used in this comparison. The first two of these agents, namely, Promin and amithiozone, showed little or no effectiveness in the dosages used. Both iproniazid and isoniazid showed appreciable degrees of activity when administered twice weekly, but were less effective than when administered daily.

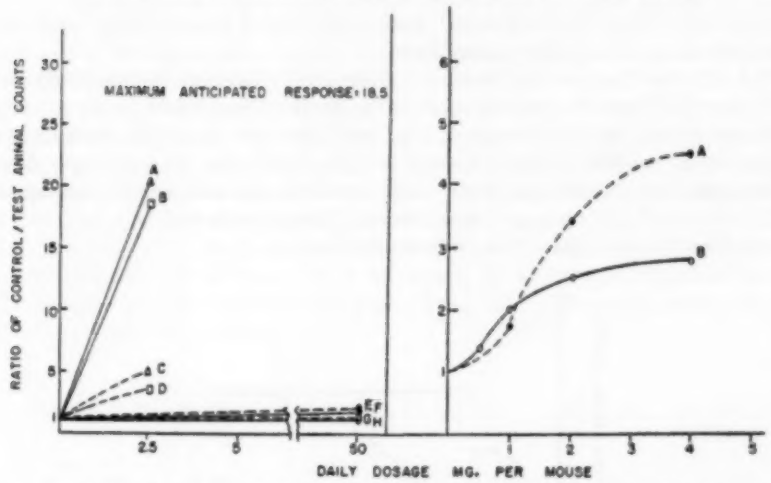

FIG. 7. Comparison of populations of *M. leprae murium* in spleen suspensions from mice receiving intermittent and daily therapy. Spleen suspensions: per cent tissue: T_{20}. Efficiency of drugs is expressed in terms of the ratio of population counts on control animals to those on treated animals.

Left: Oral therapy—counts made at 31 days.
Curve *A:* Isoniazid daily
Curve *B:* Iproniazid daily
Curve *C:* Isoniazid twice weekly
Curve *D:* Iproniazid twice weekly
Curve *E:* Amithiozone daily
Curve *F:* Amithiozone twice weekly
Curve *G:* Promin daily
Curve *H:* Promin twice weekly
Right: Subcutaneous therapy started on eighth day of infection—counts made at 29 days:
Curve *A:* Streptomycylidene isonicotinyl hydrazine daily
Curve *B:* Streptomycylidene isonicotinyl hydrazine twice weekly.
Ratio of controls at start to end of therapy = 4.5

Illustrated in figure 7*B* are the results obtained in a similar experiment in which streptomycylidene isonicotinyl hydrazine (streptomycin and isoniazid in chemical combination) was tested daily and intermittently in the control of infections in mice due to *Mycobacterium leprae murium*. Treatment was instituted in these animals on the eighth day of infection. Chemotherapeutic activity was apparent both when the drug was administered daily and when injected intermittently, e.g. twice weekly. In the single experiment conducted, 4 mg. total weight of streptomycylidene isonicotinyl hydrazine daily was adequate to prevent an increase in the number of acid-fast bacilli present in spleen suspensions at the start of therapy, but did not decrease the number of stainable organisms which had been present in such suspensions at the start of therapy. Two to four mg. per dose of the drug, administered twice weekly, were less effective than comparable amounts administered daily but more effective than 0.5 to 1 mg. admin-

istered daily. Thus, large individual doses administered intermittently appeared more effective than small frequent doses.

The effects of viomycin and isoniazid, singly and in combination, are illustrated in figure 8. Viomycin administered daily showed chemotherapeutic activity only when used in high dosage (4 mg. per mouse per day). On intermittent regimens, it was ineffective in dosages of 1, 2, and 4 mg. per mouse per dose. When administered concomitantly with isoniazid, no additive or synergistic effect was noted in the range of dosages used. There was no evidence, however, of antagonism between these two antimicrobial agents.

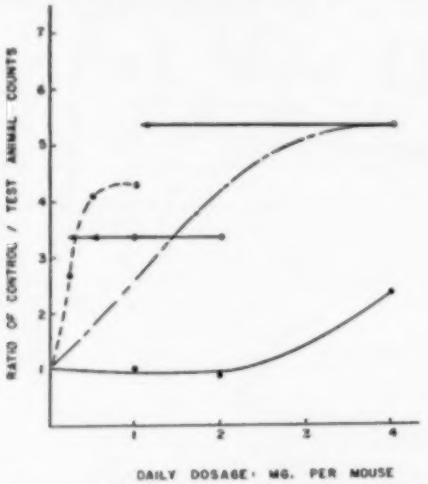

FIG. 8. Effect of viomycin and isoniazid singly and in combination upon population counts of *M. leprae murium* in spleens of mice (per cent tissue: T_{29}). Treatment started by subcutaneous route on eighth day of infection; counts made at 29 days.

●———● viomycin; ●- - - -● isoniazid; ○—·—○ viomycin plus isoniazid (plotted on basis of viomycin dosage; 0.25 mg. of isoniazid with each 1.0 gm. of viomycin; arrows indicate pull of curve to left to plot on basis of isoniazid dosage). Efficiency of drugs is expressed in terms of the ratio of population counts on control animals to those on treated animals.

Effect of interrupted therapy: In a single, so far unconfirmed, experiment, the effect of interrupted courses of therapy was tested. Ten sets of 10 mice each were infected with *M. leprae murium*. *Sets 1*, *2*, *3*, and *4* served as controls. These were sacrificed and population counts were made at 14, 36, 54, and 78 days after infection, respectively. *Sets 5*, *6*, and *7* received 1 mg. of isoniazid per mouse per day subcutaneously from the fifteenth to the thirty-fourth days of infection, at which time *Set 5* was sacrificed and population counts made. The remaining two (*Sets 6* and *7*) were held without treatment until the fifty-fourth day, at which time *Set 6* was sacrificed for population counts, while therapy was reinstituted (1 mg. per mouse per day) in *Set 7*. This last series (*Set 7*) remained on therapy until the seventy-eighth day, at which time the animals were sacrificed and population counts were again made. *Sets 8*, *9*, and *10* received 0.5 mg. of isoniazid

per mouse per day subcutaneously but, in all other respects, were treated comparably to *Sets 5*, *6*, and *7*, respectively.

As illustrated in figure 9, the chemotherapeutic effect of isoniazid which was apparent after 20 days of therapy (on the thirty-fourth day of infection) was still equally apparent after a subsequent 20-day interval without therapy (on the fifty-fourth day of infection). A second course of isoniazid, administered in a dosage of 1 mg. daily from the fifty-fourth to the seventy-eighth days of infection, resulted in a slight decrease in the number of stainable organisms. In these animals, the counts fell from 28 at 54 days to 21 at 78 days. Simultaneously, the counts in control animals rose from 119 to 1,018, thus emphasizing the degree of activity of isoniazid.

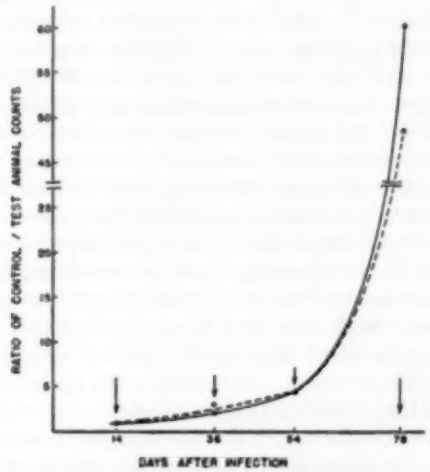

FIG. 9. Prolonged chemotherapeutic effect of isoniazid upon populations of *M. leprae murium*. Mouse spleen suspensions: per cent tissue: T_{20}. Daily therapy by the subcutaneous route started on the fourteenth day, discontinued on the thirty-sixth day, resumed on the fifty-fourth day, and discontinued on the seventy-eighth day. ●———● 1 mg. per mouse per day; ○ - - - - ○ 0.5 mg. per mouse per day. Efficiency of drugs is expressed in terms of the ratio of population counts on control animals to those on treated animals.

DISCUSSION

As mentioned previously, the development of chemotherapeutic agents for the treatment of leprosy has been handicapped to date by various factors, including the lack of sufficiently rapid and reliable drug-screening procedures. The lack of such techniques is not characteristic of this disease alone; a similar situation exists also with respect to viral, fungal, and perhaps certain of the bacterial infections. The fact that the etiologic agent of leprosy has not been cultivated to date in artificial media, the chronic nature of the disease both in animals and in man, and the lack of precise information concerning the relationship between murine and human leprosy have combined, however, to make the situation with leprosy especially difficult. The low incidence of leprosy in the United States,

in addition, has led to apathy toward studies designed to obtain the information necessary to permit development of definitive chemotherapeutic agents for its control.

In 1951, Grunberg and Schnitzer (1) reported the results of an extensive study designed to develop a refined technique for evaluation of chemotherapeutic agents against *Mycobacterium leprae murium* in mice. These investigators demonstrated the greater susceptibility of the Wiersing strain of white rats and reported that a generalized infection could be produced in mice by intraperitoneal injection of suspensions of *M. leprae murium* recovered in large quantity from such rats. The fact that the Dba strain of mice is more susceptible to the infection than the strain of white mice tested by them was also pointed out by these investigators, while data were presented to suggest that the effect of drugs upon the course of the disease could be demonstrated in such animals.

The method reported by Grunberg and Schnitzer consists of making comparative bacillary counts on localized lesions removed from the peritoneum of treated and untreated mice four weeks after intraperitoneal infection. Counts are made by crushing lesions from each animal onto microscopic slides and estimating the number of cells in such smears, after staining, by the use of a scale on which 3.0 is used to designate innumerable organisms in each microscopic field.

In the present report, a modified procedure has been described which likewise utilizes the intraperitoneal inoculation of mice with tissue suspensions from infected Wiersing rats. In this instance, however, counts have been made upon suspensions containing known weights of splenic tissue. The spleen was selected as a well-defined organ with abundant blood supply which would be heavily infected during the generalized stage of the disease. Use of an organ of this nature, rather than the localized lesions within the peritoneal cavity, seemed advisable in view of the possibility that counts made on certain lesions within a given animal might not be an accurate measure of the numbers of organisms in lesions elsewhere in the same host. Furthermore, counts on an organ of this nature seemed a better measure of the degree of infectivity during the generalized stage of the disease. The weight of splenic tissue is easily determined, and counts made on suspensions containing known weights of tissue per volume of fluid may be truly quantitative in nature. The population counts obtained from day to day, and from experiment to experiment, in the study reported herein have been remarkably reproducible and have emphasized the potentialities of the technique.

The fact that identical counts were not obtained in the single experiment conducted simultaneously in two independent laboratories and reported above emphasizes the fact that the techniques used must be identical for comparisons of this sort. The fact that the relative effectiveness of the regimens evaluated appeared the same in the two laboratories indicates the ease with which data may be compared when the technical differences are known.

The method used throughout the present study, like that of Grunberg and Schnitzer, does not differentiate between viable and nonviable cells. The population counts recorded of necessity include all stainable microbial cells. The

fact that microbial counts on spleen suspensions from animals injected with heat-treated suspensions of *M. leprae murium* have revealed bacilli in all of the experiments described in the present report suggests either that heating at 100°C. for five minutes is inadequate to sterilize or that nonviable cells may be transported to the spleen and may remain there for long periods of time. The method presented offers no means of differentiating in drug-treated animals between actively multiplying microorganisms and dormant or nonviable cells. The results obtained in a large number of animals which received graded dosages of various antimicrobial agents would indicate, nonetheless, that the relative merits of chemotherapeutic agents and of varying dosage regimens may be evaluated by this technique, provided carefully controlled conditions are used.

In a recent review, Chang and Doull (7) have called attention to the fact that interpretation of results of screening experiments conducted in recent years has proved difficult because of variation in the methods used by different investigators. Chang and Doull have summarized the conflicting results reported in the literature, and, although specifically stating that it is not advocated that all workers should adopt standard methods and protocols, have proposed that drug testing be standardized in certain respects in at least some laboratories. In considering this situation it must be recognized, however, that the use of the same dosage and route of inoculation of animals presupposes that all suspensions of *M. leprae murium* will be comparable, despite the variations which exist in the environment of different laboratories and despite differences in tissue suspensions prepared from different animals. Not all strains of mice and rats are available in all sections of the country, and strains from one breeder often are not identical with those from another breeder, even though they may pass under the same strain name. Dosage and mode of administration of drugs vary according to their pharmacologic properties and must be determined for each one tested. The period of observation furthermore must vary with the facilities of the laboratory in question. Thus, the introduction of rigid routine, even in some laboratories, may lead to false impressions concerning the effectiveness or ineffectiveness of the compounds under test, while flexibility may lead to new and valuable discoveries.

Nevertheless, one cannot overlook the conflicting data summarized by Chang and Doull (7) which pertain to the activity of antimicrobial agents against leprosy. Among the sulfones, which are generally accepted as the most effective agents in the control of this disease, apparently contradictory reports have appeared concerning the activity of 4,4′-diaminodiphenyl sulfone and Diasone. Likewise, amithiozone and also streptomycin and para-aminosalicylic acid (both widely used in the treatment of tuberculosis) have been found active by some investigators and inactive by others. Isoniazid has been reported effective against *M. leprae murium* under experimental conditions but ineffective in low dosage in the control of the disease in man (8). These results may seem confusing at first. Nevertheless, it is probable that careful evaluation of the type of disease studied, of the drug dosage used, and of the route of administration and duration of therapy might often serve to clarify some of the confusing situations. As in the case of other infectious diseases, the host-parasite relationship and the time-dose

effect of any chemotherapeutic agent cannot be ignored. A drug is effective only if it reaches the site of infection in sufficient quantity and remains there for a sufficient period of time to permit it to exert its effect upon the parasite. Small numbers of organisms circulating in a readily accessible area, such as the blood stream, may be inhibited by a smaller quantity of drug than large numbers of organisms in localized or walled-off areas. It is unfortunate indeed that many of the studies reported to date have not recorded in detail the information necessary for evaluation of the results in relation to those obtained in other studies.

In the study reported herein, evidence is presented to show that, under the experimental conditions used, isoniazid and streptomycin in combination with isoniazid (streptomycylidene isonicotinyl hydrazine) are the most effective chemotherapeutic agents available today against *M. leprae murium*. Iproniazid, streptomycin, and viomycin appear to be the next most active compounds in the control of this infection. Graded dosages have produced graded effects. Viomycin, as well as streptomycin, was active when used in combination with isoniazid; although no synergistic effect was noted, there was no evidence of antagonism among these agents.

Isoniazid in low dosage was effective when administered either daily or twice weekly. Moreover, the effect of this drug was apparent as long as three weeks after discontinuance of therapy. Animals infected 8 to 21 days prior to start of isoniazid therapy responded as well as those in which treatment was initiated on the day following infection.

The action of Promin was apparent only when large daily doses were administered. In contrast to the activity of isoniazid, the effects of Promin were generally apparent only during the first month after infection even though treatment was continued to the end of the observation period.

In the dosages used and under the experimental conditions employed in the present experiments, oxytetracycline, actithiazic acid, amithiozone, 4,4′-diaminodiphenyl sulfone, and carbomycin were ineffective.

It is of interest that quantitative differences in the action of antimicrobial agents against *M. leprae murium* were most apparent in the present experiments when treatment was continued for a period of at least 42 days after infection. Observations made 30 days after infection served only to differentiate qualitatively between active and inactive compounds.

The data presented suggest that isoniazid, either alone or in combination with streptomycin or viomycin, should be effective in the treatment of leprosy. Nonetheless, the possibility that effects observed with *M. leprae murium* in mice or rats may not be reproducible in man remains to be evaluated. Barnett and Bushby (9) have reviewed recently, however, the evidence to suggest that comparable results might be anticipated, while Hanks and Gray (10) have summarized evidence to the effect that the therapeutic effectiveness of antimetabolic drugs against less active mycobacteria may be lower and to the effect that the greater number of bacilli in established lepromatous disease may require very high dosage of drugs such as isoniazid. Thus, it seems likely that evaluation of new chemotherapeutic agents clinically will necessitate, as it has

in the case of tuberculosis, careful selection of cases in which a drug effect can be anticipated. It is not unlikely that, as in the case of tuberculosis, far advanced stages of the disease may prove relatively refractory to chemotherapy. It is not unlikely, furthermore, that lack of criteria upon which to base selection of cases for evaluation of chemotherapeutic agents, as well as lack of criteria upon which to evaluate their effects, may have combined to mask the effectiveness of at least some compounds in the clinical trials conducted to date.

In the experiments reported herein, results comparable to the *maximum anticipated response*[9] for any given time period were not achieved with isoniazid therapy when treatment was withheld until 8 to 21 days after infection. Although no increase in the number of bacilli in the isoniazid-treated animals was noted, there was likewise only a slight decrease in the number of cells during the period of observation. Whether or not the residual stainable bacilli in such animals were viable cannot be stated from the data available to date. This observation is of interest, however, in view of the fact that patients likewise may continue to harbor stainable bacilli after therapy which cannot be tested for viability because of the present inability either to culture *M. leprae murium* or to transmit the infection from man to animals.

The greater activity of isoniazid and, to a lesser extent, that of streptomycin, in contrast to that of Promin and the commonly accepted sulfones, in experimental infections produced by *M. leprae murium* has been reported also by Barnett and Bushby (9) and by Chang (11). It is conceivable, and indeed highly probable, that the relative activities of these compounds may be related, at least in part, to the relative ease with which they diffuse through cell membranes and to the degree of activity which they are capable of exerting in an intracellular environment. Although no reports have appeared to date to indicate whether or not the sulfones are active intracellularly, evidence has been presented to show that isoniazid is as effective intracellularly as extracellularly and that streptomycin is partially effective under intracellular conditions, whereas para-aminosalicylic acid is ineffective under such conditions (12, 13).

It is difficult to predict the type of compound which might be effective as a chemotherapeutic agent against any infectious disease. Experience in the field of chemotherapeutic agents in general, however, leads to the suspicion that a satisfactory drug against leprosy would have the following properties:

(*1*) It must be capable of inhibiting growth of mycobacteria.
(*2*) It must be effective in the presence of body fluids and tissue.
(*3*) It must be active against large masses of slowly multiplying microorganisms.
(*4*) It must be distributed readily throughout the body of the host and must be capable of passing through cell membranes and remaining effective in an intracellular environment.
(*5*) It must possess a sufficiently low degree of toxicity to permit administration over prolonged periods of time.

[9] *The maximum anticipated response* is defined as the ratio between the population counts on spleen suspensions of control animals and the counts on spleen suspensions of animals injected with heat-treated bacilli (100°C.—5 minutes).

Unless the Hawaiian strain of *Mycobacterium leprae murium* differs significantly from strains of *Mycobacterium leprae* which produce disease in humans, there seems little reason to believe that drugs fulfilling these five criteria and exerting a demonstrable effect against *Mycobacterium leprae murium* in an infection such as is described in the present report should not be effective against the disease in man.

SUMMARY

A procedure has been described whereby the chemotherapeutic action of antimicrobial agents against *M. leprae murium* may be evaluated rapidly. The reproducibility of results obtained by this procedure and the possible parallel between such results and those which might be attained in man have been discussed. Data have been presented to show that, under the experimental conditions used in this study, isoniazid and streptomycin in combination with isoniazid (streptomycylidene isonicotinyl hydrazine) are the most effective agents available against *M. leprae murium*. Iproniazid, streptomycin, and viomycin, respectively, were the next most effective agents. All of the five compounds were active in lower dosages and over more prolonged periods of time than Promin. Factors influencing antimicrobial activity *in vivo* and certain criteria which may be essential to an effective chemotherapeutic agent against leprosy are discussed.

SUMARIO

Justipreciación de los Agentes Quimioterapéuticos en el Dominio de las Infecciones Experimentales Debidas al Mycobacterium Leprae Murium

El procedimiento descrito permite valuar rápidamente la acción quimioterapéutica de los agentes antimicrobianos contra el *M. leprae murium*. Discútense la reproducibilidad de los resultados obtenidos con dicho procedimiento y el posible paralelo entre los mismos y los que podrían alcanzarse en el hombre. Preséntanse datos para demostrar que, en la situación experimental usada en este estudio, la isoniacida y la combinación de isoniacida y estreptomicina (hidracina isonicotinilica estreptomicilidénica) son los agentes más eficaces que haya disponibles contra el *M. leprae murium*, viniendo después la iproniacida, la estreptomicina y la viomicina, respectivamente. Todos estos cinco compuestos de mostraron activos a dosis menores y durante perfodos de tiempo más prolongados que la Promina. Discútense los factores que afectan la actividad antimicrobiana *in vivo* y ciertas propiedades que tal vez sean indispensables en un agente quimioterapéutico eficaz contra la lepra.

RESUME

Une évaluation des agents chimiothérapeutiques dans la lutte contre l'infection expérimentale par la Mycobacterium leprae murium

Description d'un procédé permettant d'évaluer rapidement l'action chimiothérapeutique des agents antibactériens sur le *M. leprae murium*. La possibilité de reproduire les résultats fournis par cette méthode et le parallélisme possible entre ces résultats et ceux pouvant être atteints chez l'homme sont examinés. Les auteurs présentent des données démontrant que, dans les conditions expérimentales de cette étude, l'isoniazide et l'isoniazide associeé à la streptomycine (streptomycylidene isonicotinyl hydrazine) sont les agents les plus efficaces contre le *M. leprae murium*. Viennent immédiatement ensuite par ordre d'efficacité: l'iproniazide, la streptomycine et la viomycine. Ces cinq composés sont actifs à dose plus

faible et pendant des périodes plus prolongées que la Promine. Les facteurs influençant l'activité antibactérienne *in vivo* et certains critères considérés essentiels pour un agent chimiothérapeutique efficace contre la lèpre sont examinés.

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TUBERCULOUS MENINGITIS[1]

Treatment with Streptomycin, Para-Aminosalicylic Acid and Promizole, Isoniazid and Streptomycin, and Isoniazid

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INTRODUCTION

Meningitis caused by *Mycobacterium tuberculosis* has long been recognized as one of the most serious infections of the central nervous system. In the last ten years, since streptomycin first became available, this drug alone and in combination with other antituberculous agents, such as para-aminosalicylic acid (PAS) and Promizole, has been used with varying degrees of success (1–8). More recently, isoniazid has been successfully employed in a small number of patients with tuberculous meningitis (9–14).

Since there is a considerable variation in the percentages of patients in whom prolonged remission is reported following the use of chemotherapy, it is difficult to compare the results of any two therapeutic regimens when the patients have been treated in different clinics. Accordingly, the present writers are reporting the results seen in 87 patients treated on the same service, 61 with streptomycin and an antituberculous drug other than isoniazid, 13 with isoniazid alone, and 13 with isoniazid plus streptomycin.

METHOD

The patients were treated on the wards of the Municipal Contagious Disease Hospital of Chicago. As this institution received a large proportion of the patients with acute meningitis from the city of Chicago and admitted virtually no tuberculous patients except those with meningitis, most of these patients were admitted with advanced and acute involvements of the nervous system.

In almost all instances, treatment was started for tuberculous meningitis in a patient with a rapidly deteriorating condition who had a cerebrospinal fluid showing a low cell count, usually with a predominance of lymphocytes, a low dextrose, and a high protein concentration. For this reason each of the patients was started on treatment between the first and eighth days of hospitalization whether or not positive proof of the identity of the infection had been obtained. Nevertheless, in all of the patients reported in this paper except one, the diagnosis was later proved to have been tuberculosis either by a positive culture or guinea pig inoculation or by post-mortem examination. The one exception was a patient in *Group IV*, as defined below, who has remained well for twenty-one months after streptomycin therapy was discontinued and in whom all clinical criteria pointed to a diagnosis of tuberculosis. Inclusion of this case is consistent

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with our finding that the clinical diagnosis of tuberculosis was confirmed by post-mortem examination in all of the patients on whom this examination was made although cultures of cerebrospinal fluid and other fluids were negative in 10 per cent of those patients during life.

Drugs: The drugs and doses used were as follows: (*1*) Streptomycin sulfate or dihydrostreptomycin sulfate was given intramuscularly, 25 mg. per kg. per day up to a maximum dose of 2 gm. per day, divided into three or four doses. In addition, streptomycin sulfate was given intrathecally in doses of 100 mg., except in infants under one year of age who received only 50 mg. per injection. (*2*) Para-aminosalicylic acid was given by mouth or intubation in doses of 150 to 200 mg. per kg. for each twenty-four-hour period in divided doses, or the maximum tolerated dose if it was less than this. When the drug was given intravenously as a continuous 2 per cent infusion, the dose was 500 mg. per kg. for a twenty-four-hour period, with a maximum dose of 40 gm. (*3*) The initial dose of Promizole was 100 mg. per kg. for each twenty-four-hour period orally or by intubation. Subsequent doses were administered so as to maintain the blood Promizole concentration between 1 and 4 mg. per 100 ml. (*4*) Isoniazid[2] was administered in doses of 5 mg. per kg. for each twenty-four-hour period to the first 16 patients. In 6 of the last 10 patients, the dose was increased to 8 mg. per kg. for each twenty-four-hour period when the progress appeared to be poor. It was administered intramuscularly in most of the patients for at least one to three weeks and, thereafter, by mouth or stomach tube. Chemical assay of the concentration of isoniazid in the blood and cerebrospinal fluid was made by the method of Kelly and Poet (15). The studies confirmed free diffusion into the cerebrospinal fluid but otherwise were not used as a guide to therapy.

Regimens: The 61 patients treated during the first two years of these studies were divided into four treatment groups as follows:

Group I. Four patients received streptomycin alone. Two of these received intrathecal therapy and 2 did not. Of those who received the intrathecal treatment, one received injections on alternate days. The second patient received daily injections for forty-five days and then on alternate days for another ninety days.

Group II. Nineteen patients received intramuscular streptomycin or dihydrostreptomycin and oral Promizole. Ten of these received no intrathecal treatment, and 9 were given streptomycin intrathecally every second or third day.

Group III. Seventeen patients were given intramuscular streptomycin or dihydrostreptomycin and oral Promizole. In each instance the patient received, in addition, daily intrathecal medication for forty-five days, followed by at least 45 more injections on alternate days, unless death interrupted treatment.

Group IV. Twenty-one patients were treated with streptomycin intramuscularly and intrathecally, as in *Group III.* In addition, all received PAS orally or intravenously and 10 also received Promizole.

It should be emphasized that the patients in these groups were not alternated but that many were treated concurrently in a random fashion.

In the past year since isoniazid first became available, the patients have been assigned alternately between two groups. The first, referred to as *Group V* in this report, received streptomycin intramuscularly and intrathecally and isoniazid intramuscularly or orally. Patients in the other group (*Group VI*) were treated with isoniazid as the sole antimicrobial agent.

Other measures used, besides symptomatic treatment, were the intrathecal instillation of tuberculin by the method of Smith and Vollum (6), or of streptokinase and strepto-

[2] The isoniazid used in this study was supplied as Rimifon by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

dornase, and surgical decompression. These were used on patients who developed evidence of a block to the circulation of cerebrospinal fluid. The patients so treated were distributed among all of the groups except *Group I*. It has been impossible to evaluate the worth of these procedures since their use was restricted to patients with poor prognoses.

In describing results in this paper, the term *remission* has been used to designate survival for six months after the start of treatment, and *prolonged remission* for those patients who have been alive and essentially well six months after the therapy was discontinued.

RESULTS

Survival in all groups: As shown in table 1, 61 patients were treated before the introduction of isoniazid. Only one of the 4 patients in *Group I* survived six months. As this patient tolerated neither PAS nor Promizole, she had been treated with streptomycin alone, including daily intrathecal instillations for forty-five days followed by 45 injections on alternate days. In spite of the good initial response to treatment, this patient's meningitis relapsed in the eighth month and she was treated with isoniazid in another institution.

Among the 19 patients in *Group II*, only one survived for six months after the start of treatment; this patient is still alive and apparently well thirty-

TABLE 1

CONDITION OF PATIENTS AFTER RECEIVING DIFFERENT CHEMOTHERAPEUTIC REGIMENS FOR SIX MONTHS

GROUP	DRUG	ROUTE	NUMBER OF PATIENTS	ALIVE SIX MONTHS AFTER START OF TREATMENT	
				Number	Per Cent
I	Streptomycin	I.M. and I.T. or D.I.T.	4	1*	25.0
II	Streptomycin Promizole	I.M. +/− I.T. p.o.	19	1	5.2
III	Streptomycin Promizole	I.M. and D.I.T. p.o.	17	5†	29.4
IV	Streptomycin PAS with or without Promizole	I.M. and D.I.T. p.o. or i.v.	21	4‡	19.0

I.M. = intramuscularly
I.T. = intrathecally every second or third day
D.I.T. = intrathecally daily for at least 45 days
p.o. = orally
i.v. = intravenously

* Patient's meningitis relapsed in the eighth month.

† Two of these patients were in poor condition at the end of six months and subsequently died. One patient's meningitis relapsed 14 months after discontinuing therapy.

‡ One of these patients experienced a relapse 18 months after discontinuing therapy.

five months later. On the other hand, 5 of 17 of the patients in *Group III* were alive at six months, and 3 had prolonged remissions. Four of the 21 patients in *Group IV* had remissions lasting more than six months. Ten of the patients in *Group IV* received a continuous intravenous infusion of 2 per cent sodium PAS in doses up to 40 gm. a day in adults and 500 mg. per kg. per day in children. Only one of these patients has had a remission. One of the patients in *Group III* subsequently experienced a relapse fourteen months after the termination of streptomycin and PAS therapy, which he received for nine months. The meningitis of one of the patients in *Group IV* relapsed eighteen months after streptomycin and PAS therapy was stopped and five months after Promizole was discontinued. This patient had received streptomycin and PAS for eight months and Promizole for twenty-one months.

Intrathecal therapy: As shown in table 1 and summarized in table 2, the most important single factor in producing remissions in these patients was the use of daily intrathecal streptomycin treatment initially, followed by treatment at least every other day for 135 days or more from the onset of treatment. Analysis of age, sex, race, distribution, incidence of miliary disease, and degree of illness and disability revealed the patients to be equally distributed between the group which received daily intrathecal therapy and the group in which intermittent or no intrathecal medication was given.

Since the best results prior to the introduction of isoniazid had been in the patients who received daily intrathecal and intramuscular streptomycin with oral or intravenous PAS and/or Promizole, all of the patients who received this type of treatment (*Groups III* and *IV*) have been added together for comparison with the groups of patients who received isoniazid.

As may be seen in table 3, the results with isoniazid alone (*Group VI*) were indistinguishable from those obtained with isoniazid plus intrathecal and intramuscular streptomycin (*Group V*). In both groups, the results were superior to those obtained prior to the use of isoniazid.

Isoniazid regimens compared with other therapies: If the results in the 26 patients who have been treated with isoniazid with or without streptomycin (*Groups V*

TABLE 2

EFFECT OF DAILY INTRATHECAL STREPTOMYCIN TREATMENT OF PATIENTS WITH TUBERCULOUS MENINGITIS

TYPE OF INTRATHECAL THERAPY	NUMBER OF PATIENTS	ALIVE SIX MONTHS AFTER START OF TREATMENT	
		Number	Per Cent
None	9	0	0.0
Every second or third day	13	1	7.7
Daily for at least 45 days	39	10*	25.6

* Two patients were in poor condition at the end of six months and subsequently died. Three patients experienced relapses, one each at 2, 14, and 18 months after therapy was discontinued. Five (12.9 per cent) were alive and apparently well from 8 to 25 months after cessation of therapy

TABLE 3

RESULTS OF TREATMENT OF TUBERCULOUS MENINGITIS BEFORE AND AFTER THE INTRODUCTION OF ISONIAZID

GROUP	DRUG	ROUTE	NUMBER OF PATIENTS	ALIVE SIX MONTHS AFTER START OF TREATMENT	
				Number	Per Cent
III and IV	Streptomycin Promizole and/or PAS	I.M. and D.I.T. p.o. p.o. or i.v.	38	9*	23.6
V	Isoniazid Streptomycin	I.M. or p.o. I.M. and D.I.T.	13	7†	53.8
VI	Isoniazid	I.M. or p.o.	13	7†	53.8

* Two patients in poor condition at six months.

† One patient in poor condition at six months.

and *VI*) are compared with the results obtained in the groups with the lowest fatality rates before the advent of isoniazid (*Groups III* and *IV*), the 23.6 per cent remission rate of the former groups is significantly lower than the 53.8 per cent remission rate for the latter when tested by the chi-square method (chi-square = 4.0).

Age and race distributions: In table 4, it is shown that the age and race distributions of patients among *Groups III* and *IV*, *Group V*, and *Group VI* were approximately the same. Apparently the lower fatality rate achieved with the use of isoniazid was not attributable to any unusual distribution of patients. Moreover, it can be seen that isoniazid has not improved the prognosis in infants less than two years of age, but has been most effective in the two- to twenty-year age group (90 per cent remission rate) and moderately effective in the adult group, in which there was a 50 per cent remission rate. Isoniazid has been quite successful in the treatment of the nonwhite patients in contrast to rather poor results prior to the introduction of isoniazid.

Factors related to severity of infection: Certain factors related to the severity of the disease are shown in table 5. The percentage of patients with miliary disease was slightly lower in the isoniazid groups than in the patients treated prior to the advent of isoniazid. The same was true of the percentage of patients who had a clear sensorium and only meningitic signs at the time treatment was started. Moreover, the results of isoniazid treatment of patients with miliary as well as meningeal tuberculosis were better than the results obtained with streptomycin.

That approximately one-fourth of the patients in each of the treatment groups died in the first week of treatment is shown in table 6. In those patients who survived the first week of therapy, the prognosis was much better in those treated with isoniazid. The efficacy of isoniazid is illustrated by the fact that, among the patients in *Groups V* and *VI* alive at the end of one week of treatment, only 3 of 19 (15.6 per cent) died during the ensuing three weeks, whereas among the

TABLE 4

AGE AND RACE DISTRIBUTION AMONG TUBERCULOUS MENINGITIS PATIENTS IN DIFFERENT THERAPEUTIC GROUPS

GROUP	DRUG	ROUTE	TOTAL IN GROUP	PROGNOSTIC SIGNS																	
				Age in Years												Race					
				Less than 2				2 to 20				More than 20				White				Nonwhite			
				Total		Lived		Total		Lived		Total		Lived		Total		Lived		Total		Lived	
				Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
III and IV	Streptomycin	I.M. and D.I.T.	38	13	34.2	2*	15.4	14	36.8	4*	28.6	11	29.0	3	27.3	17	44.8	5	29.4	21	55.2	4†	19.0
	Promizole and/or PAS	p.o. p.o. or i.v.																					
V	Isoniazid Streptomycin	I.M. or p.o. I.M. and D.I.T.	13	3	23.1	0	0.0	5	38.4	4*	80.0	5	38.4	3	60.0	8	61.5	3*	37.5	5	38.5	4	80.0
VI	Isoniazid	I.M. or p.o.	13	3	23.1	0	0.0	5	38.4	5*	100.0	5	38.4	2	40.0	6	46.1	3*	50.0	7	53.9	4	57.1

Lived = alive at six months.

* One patient in poor condition at six months of treatment.

† Two patients in poor condition at six months of treatment.

TABLE 5

CLINICAL CONDITION OF PATIENTS WITH TUBERCULOUS MENINGITIS IN DIFFERENT THERAPEUTIC GROUPS AT THE START OF TREATMENT

GROUP	DRUG	ROUTE	TOTAL IN GROUP			PATIENTS WITH GENERALIZED MILIARY DISEASE				PATIENTS WITH CLEAR SENSORIUM AND ONLY MENINGITIC SIGNS			
				Alive at 6 Months				Alive at 6 Months				Alive at 6 Months	
			Number	Number	Per Cent	Number	Per Cent of Total	Number	Per Cent	Number	Per Cent of Total	Number	Per Cent
III and IV	Streptomycin Promizole and/or PAS	I.M. and D.I.T. p.o. p.o. or i.v.	38	9*	23.6	18	47.3	2	11.1	8	21.5	2	25.0
V	Isoniazid Streptomycin	I.M. or p.o. I.M. and D.I.T.	13	7†	53.8	4	30.8	2	50.0	2	15.4	2	100.0
VI	Isoniazid	I.M. or p.o.	13	7†	53.8	5	38.4	3†	60.0	2	15.4	2	100.0

* Two patients in poor condition at the end of six months.
† One patient in poor condition at the end of six months.

TABLE 6

INTERVAL BETWEEN START OF TREATMENT AND DEATH IN PATIENTS DYING FROM TUBERCULOUS MENINGITIS

GROUP	DRUG	ROUTE	TOTAL NUMBER OF PATIENTS TREATED	PATIENTS WHO DIED AT THE FOLLOWING INTERVALS									
				1 Week		1 Week to 1 Month		1 to 2 Months		2 to 3 Months		4 to 6 Months	
				Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
III and IV	Streptomycin Promizole and/or PAS	I.M. and D.I.T. p.o. p.o. or i.v.	38	10	26.3	14	37.4	2	5.3	0	0	3	7.9
V	Isoniazid Streptomycin	p.o. or I.M. I.M. and D.I.T.	13	3	23.1	2	15.4	1	7.7	0	0	0	0.0
VI	Isoniazid	p.o. or I.M.	13	4	30.8	1	7.7	1	7.7	0	0	0	0.0

patients in *Groups III* and *IV*, 14 of 28 (50 per cent) died during the corresponding period.

Cerebrospinal fluid findings: Among the patients who experienced a remission, the temperature, the leukocyte count, and the glucose and protein content of the cerebrospinal fluid tended to reach normal limits in patients in *Groups V* and *VI* more rapidly after the start of treatment than in patients in *Groups III*

TABLE 7

RAPIDITY OF RESPONSE TO TREATMENT IN PATIENTS WITH TUBERCULOUS MENINGITIS WHO SURVIVED

INDEX OF IMPROVEMENT	GROUP	DRUG	ROUTE	NUMBER OF PATIENTS WITH COMPLETE REMISSION	1 Week		1 Week to 1 Month		1 Month to 2 Months		2 Months to 3 Months		3 Months to 6 Months		6 Months	
					Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Rectal temperature consistently below 100°F.	III and IV	Streptomycin Promizole and PAS	I.M. and D.I.T. p.o. p.o. or i.v.	7	0	0	0	0	2	28.6	1	14.3	3	42.8	1	14.3
	V	Isoniazid and streptomycin	I.M. or p.o. I.M. and D.I.T.	7	0	0	5	71.4	1	14.3	0	0	0	0	1	14.3
	VI	Isoniazid	I.M. or p.o.	7	1	14.3	5	71.4	0	0	0	0	0	0	1	14.3
Cerebrospinal fluid dextrose consistently above 50 mg. per 100 ml.	III and IV	Streptomycin Promizole and PAS	I.M. and D.I.T. p.o. p.o. or i.v.	7	1*	14.3	0	0	0	0	2	28.6	3	42.8	1	14.3
	V	Isoniazid and streptomycin	I.M. or p.o. I.M. and D.I.T.	7	2	28.6	2	28.6	1	14.3	0	0	1	14.3	1	14.3
	VI	Isoniazid	I.M. or p.o.	7	0	0	2	28.6	2	28.6	1	14.3	1	14.3	1	14.3
Cerebrospinal fluid protein consistently below 40 mg. per 100 ml.	III and IV	as above		7	0	0	0	0	0	0	0	0	1	14.3	6	88.7
	V	as above		7	0	0	0	0	0	0	1	14.3	2	28.6	4	57.1
	VI	as above		7	0	0	1	14.3	1	14.3	2	28.6	1	14.3	2	28.6
Cerebrospinal fluid cell count consistently below 5 per cu.mm.	III and IV	as above		7	0	0	0	0	0	0	0	0	2	28.3	5	71.7
	V	as above		7	0	0	0	0	1	14.3	1	14.3	1	14.3	4	57.1
	VI	as above		7	0	0	1	14.3	0	0	1	14.3	1	14.3	4	57.1

* One never below 50 mg. per 100 ml.

and *IV*. There was little difference between the patients who received isoniazid alone and those who received isoniazid plus streptomycin except that the protein content of the cerebrospinal fluid in the former group usually reached a normal level sooner. This may be an indication of irritation by the intrathecal medication rather than a delayed response to therapy.

Since the course of the pleocytosis has been stated to have prognostic significance in streptomycin-treated patients (9), the following observations have been made. Inasmuch as the cell count on successive days (even without treatment) is quite variable, a significant rise was considered to be present only when the count rose more than 50 cells per cu. mm. Ten patients in *Groups III* and *IV* showed such an increase, and 3 of them attained a satisfactory remission of their meningitis. On the other hand, 15 patients exhibited no such increase, and 4 of these experienced remissions. Moreover, among the 7 patients with remissions in *Group V*, 4 showed no rise in the cerebrospinal fluid cell count; whereas among the 7 patients with a remission in *Group VI*, 4 likewise showed no increase of cells in the fluid.

Residual neurologic defects: Residual neurologic defects have been numerous. Among the 7 patients in *Groups III* and *IV* who attained satisfactory remissions, one was deaf, 2 had hemiplegia, and 2 had some leg weakness and pain over the distribution of the sciatic nerve. Among the 12 patients in *Groups V* and *VI* who attained satisfactory remissions, 3 have partial cranial nerve palsies and one has weakness in one leg.

Drug toxicity: In addition to the patient with deafness, who received dihydrostreptomycin, 6 patients who received streptomycin had mild to moderate disturbances of equilibrium. Two patients developed fever and rash related to PAS administration. Although most of the older patients who regained consciousness exhibited a mild euphoria during isoniazid administration, only one serious reaction occurred with this drug; this was a definite mania which required temporary interruption of treatment in the fifth month. The only reaction attributable to Promizole was rash accompanied by fever in one patient.

DISCUSSION

The prognosis in tuberculous meningitis is so intimately related to the age and general condition of the patients treated and the duration and severity of the illness at the time therapy is started that it is difficult to compare results obtained in one hospital with those observed in other hospitals.

These and other less tangible factors are best controlled by observations in a single clinic where the management of the disease is the same except for the specific drug regimens used, as was the case with the patients in the present report.

Although patients who received intrathecal streptomycin every day were not treated in strict alternation with those who received less intrathecal streptomycin or none at all by this route, the types of patients treated and the severity of the disease were comparable in the different groups.

Accordingly, the superior results obtained in the patients who received strepto-

mycin intrathecally at daily intervals for at least forty-five days can be attributed to the more vigorous intrathecal therapy. As a matter of fact, until the introduction of isoniazid, the intrathecal route of streptomycin administration was related to a successful outcome much more closely than any other variable in treatment, such as the choice of the agent used as an adjuvant to streptomycin.

The superior results obtained in the patients treated with isoniazid to those obtained previous to its introduction also appear to be due to the specific therapy employed even though the patients were not treated in alternation. The results are similar to those reported by Clark and his associates in a smalier number of patients (9).

Since the results obtained with isoniazid alone up to the present time have been as good as those obtained with isoniazid plus intramuscular and intrathecal streptomycin, it is possible that streptomycin need not be given in addition. Before a definite conclusion can be reached, however, a larger number of patients must be followed for a longer period of time after the cessation of therapy. It is likely, however, that the penetration of isoniazid into the cerebrospinal fluid, reported by Elmendorf and co-workers (16) and confirmed in the present study, will obviate the necessity for intrathecal streptomycin therapy in the future. Whether intramuscular streptomycin should be used in addition to isoniazid will be determined by the frequency of relapses and, particularly, whether isoniazid-resistant tubercle bacilli are responsible for the relapses.

Analysis of the individual factors of age, race, the presence of miliary disease, and the degree of nervous system involvement showed that there was no weighting in favor of the isoniazid-treated patients. The most disappointing results are still those obtained in patients with severe neurologic difficulty at the start of treatment, particularly in those less than two years of age.

SUMMARY

Sixty-one patients with tuberculous meningitis were treated before isoniazid was available, and among these only 10 survived for six or more months. All of the patients who experienced a remission of their meningitis received streptomycin, and all but one received para-aminosalicylic acid (PAS) and/or Promizole in addition. The choice of whether PAS, Promizole, or both were used in addition to streptomycin had little effect on the outcome. However, daily intrathecal treatment with streptomycin for the first forty-five days did seem important. Among 38 patients who received streptomycin intramuscularly and by daily intrathecal instillations with PAS and/or Promizole in addition, 9 (26.3 per cent) survived six months or more.

Thirteen patients with tuberculous meningitis were treated with isoniazid alone and 13 with isoniazid plus intramuscular and daily intrathecal instillations of streptomycin. Seven patients in each group lived for six months or more. These results are significantly better than those obtained when streptomycin was used without isoniazid. The results obtained with isoniazid were as good as those obtained in patients treated with isoniazid plus intramuscular streptomycin.

Age, race, the presence of miliary disease, and degree of involvement of the

nervous system influence prognosis greatly. These factors were uniformly distributed in the groups under discussion. Results of treatment with isoniazid were better than the results obtained with streptomycin in the absence of isoniazid in all types of patients and regardless of the severity of the disease except in patients less than two years of age, in whom remissions were extremely rare.

Clinical improvement was more rapid in patients who received isoniazid.

Residual neurologic sequelae were less frequent in the isoniazid-treated patients.

Toxic reactions to a drug were most frequent in patients who received streptomycin or dihydrostreptomycin and were infrequently observed as a result of treatment with para-aminosalicylic acid, Promizole, or isoniazid.

SUMARIO

Meningitis Tuberculosa. Tratamiento con Estreptomicina, Promizol y Acido Para-Aminosalicílico, Isoniacida y Estreptomicina e Isoniacida

Sesenta y un enfermos con meningitis tuberculosa fueron tratados antes de contarse con la isoniacida y, de ellos, sólo 10 sobrevivieron seis meses o más. Todos los que tuvieron remisiones recibieron estreptomicina y todos, menos uno, recibieron ácido para-aminosalicílico y/o Promizol además. El hecho de usarse PAS, Promizol o ambos, además de la estreptomicina, afectó poco el resultado. Sin embargo, pareció importante el tratamiento intratecal diario con estreptomicina durante los primeros cuarenta y cinco días. De 38 enfermos que recibieron estreptomicina intramuscularmente y en instilaciones intratecales diarias con PAS y/o Promizol además, 9 (26.3 por ciento) sobrevivieron seis meses o más.

A 13 enfermos de meningitis tuberculosa se les trató con isoniacida sola y a 13 con isoniacida junto con estreptomicina intramuscularmente y en instilaciones intratecales diarias. Siete enfermos de cada grupo vivieron seis meses o más. Esos resultados son decididamente mejores que los obtenidos cuando se usaba la estreptomicina sin isoniacida. Los resultados conseguidos con la isoniacida sola fueron tan buenos como los obtenidos en los enfermos tratados con isoniacida y además estreptomicina intramuscularmente.

La edad, la raza, la presencia de granulia y la intensidad de la invasión del sistema nervioso afectan considerablemente el pronóstico. En los grupos aquí considerados, esos factores acusaron una distribución uniforme. Los resultados del tratamiento con isoniacida fueron mejores que los obtenidos con estreptomicina sin isoniacida en los enfermos de todos géneros e independientemente de la gravedad de la dolencia, excepto en los menores de dos años, en quienes las remisiones fueron sumamente raras.

La mejoría clínica fué más rápida en los enfermos que recibieron isoniacida.

Las secuelas neurológicas residuales fueron menos frecuentes en los tratados con isoniacida.

Las reacciones tóxicas alcanzaron su mayor frecuencia en los enfermos que recibieron estreptomicina o dihidroestreptomicina, observándose raramente a consecuencia del tratamiento con ácido para-aminosalicílico, Promizol o isoniacida.

RESUME

Méningite tuberculeuse. Traitement par la streptomycine, le Promizole et l'acide para-aminosalicylique, par l'isoniazide et la streptomycine, ou par l'isoniazide

Soixante et un cas de méningite tuberculeuse ont été traités avant que l'on ne dispose de l'isoniazide et 10 de ces malades seulement ont survécu six mois et plus. Tous ceux des malades présentant une rémission ont reçu de la streptomycine et tous, sauf un, ont reçu en plus de l'acide para-aminosalicylique et du Promizole ou l'un d'eux. L'adjonction de PAS, de Promizole ou de l'un et l'autre de ces deux agents ne modifiait guère l'issue. D'autre

part, le traitement journalier par la streptomycine par voie sous-arachnoïdienne pendant les quarante-cinq premiers jours a paru important. Parmi les 38 patients qui ont été traités par la streptomycine intramusculaire associée à l'instillation sous-arachnoïdienne quotidienne de PAS et de Promizole ou de l'un ou l'autre de ces agents, 9 d'entre eux (26,3%) survivaient six mois ou plus.

Treize malades affectés de méningite tuberculeuse ont été traités par l'isoniazide seule et 13 ont été traités par l'isoniazide avec adjonction quotidienne de streptomycine intramusculaire et sous-arachnoïdienne. Sept des malades de chaque groupe ont survécu pendant six mois ou plus. Ces résultats ont une valeur nettement supérieure à ceux qui ont été obtenus quand la streptomycine était administrée sans isoniazide. Les résultats obtenus avec l'isoniazide sont aussi bons que ceux obtenus chez les malades recevant l'isoniazide plus la streptomycine intramusculaire.

L'âge, la race, la présence d'une miliaire et le degré de l'altération du système nerveux influencent profondément le pronostic. Ces divers facteurs étaient uniformément répartis dans les groupes étudiés.

Les résultats du traitement par l'isoniazide se sont montrés meilleurs que ceux obtenus avec la streptomycine sans adjonction d'isoniazide dans toutes les catégories de malades et quelle que soit la sévérité du cas, sauf chez les malades âgés de moins de deux ans où les rémissions observées furent extrêmement rares.

L'amélioration clinique s'est manifestée plus rapidement chez les malades traités par l'isoniazide.

Les séquelles neurologiques étaient moins fréquentes dans le groupe traité par l'isoniazide.

Les réactions toxiques étaient plus fréquemment observées chez les malades qui recevaient la streptomycine ou la dihydrostreptomycine, elles étaient rarement constatées à la suite du traitement par l'acide para-aminosalicylique, le Promizole ou l'isoniazide.

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STUDIES ON NONSPECIFIC HYPERERGIC REACTIVITY AT THE SITE OF THE TUBERCULIN REACTION[1, 2]

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INTRODUCTION

The observation that apparently there is a parallel development of both specific and nonspecific hyperergy[3] in tuberculous guinea pigs, as measured by the amount of an intravenous provoking agent required to elicit a lethal systemic reaction, suggests that these two types of changed reactivity are only different manifestations of one and the same fundamental change (2). There are, however, deviations from this parallel. For example, the fully developed nonspecific hyperergy of tuberculous guinea pigs represents only an approximate eightfold increase in response to the provoking agent when compared with the susceptibility of nontuberculous control pigs, whereas the systemic tuberculin sensitivity rises from nearly zero. Among others, Bordet, Shwartzman, Rich, and Packalén (1–5) have discussed whether the nonspecific hyperergic reactions might depend upon the mechanism of the Shwartzman phenomenon. To date, however, no conclusive evidence has been produced, due possibly, and in part, to the inability to estimate exactly the degree of hyperergic reactivity. Owing to difficulties in quantitative estimation, an evaluation of hyperergic reactivity by observing focal reactions develop at tuberculous sites after provocation (3, 4, 6–8) is likely to give no better results than by recording systemic reactions. On the other hand, post-provocation hemorrhagic necrotic focal reactions which appear at the sites of tuberculin skin tests (5, 7, 9) seem to offer better opportunities for quantitative studies. It may be mentioned in this connection that a method for precise numerical estimation has been developed by Long and Miles (10) on the basis of their own and Wadley's (11) discovery of the linear relationship between the log-dose of tuberculin and the lesion diameter of the Mantoux test.

In producing the basic Shwartzman phenomenon, usual procedure involves two separate injections of the reactive agents, the intradermal local preparation and the intravenous provocation. Recently, however, Black-Schaffer and associates (12) demonstrated the production of a Shwartzman-like cutaneous reaction by means of a single intradermal injection. This result could be achieved

[1] From the Bacteriological Laboratory and the Department of Medical Tuberculosis, St. Göran's Hospital, and the Central Municipal Bacteriological Laboratory, Stockholm, Sweden.

[2] The expenses of this study were defrayed by grants from the Swedish National Association Against Tuberculosis.

[3] When speaking of, or including in the discussion of specific hypersensitivity, nonspecific reactions of the types studied, the writers have preferred the use of "hyperergy" and "hyperergic reactivity" to "hypersensitivity" as a wider and noncommital term, reserving the latter for the well-established types of specifically increased reactivity—the tuberculin, anaphylactic, pollen, Arthus types of hypersensitivity (1).

if an agent to which the experimental animal had been rendered hypersensitive was added to the preparatory inoculum of toxin. These investigators were not inclined to consider the resulting reaction as simply the summation of two injurious factors, but believed, instead, that in these circumstances the Shwartzman reaction functions as an amplifier of the antigen-antibody tissue response.

The present report is concerned with nonspecific hyperergic reactivity to intravenous or local provocation of the tuberculin site throughout its various stages of development and with some of the divergencies between specific and nonspecific hyperergy noted during the study.

MATERIALS AND METHODS

Male guinea pigs weighing between 250 and 350 gm. were infected in the thigh by a subcutaneous injection of 0.5 ml. of a 1:100 dilution of an eight-day Dubos (fluid medium) culture of the H37Rv strain of *M. tuberculosis*. Prior to the main experiments, five to seven weeks later, the guinea pigs were divided into groups comprising 4 to 11 animals, each of approximately the same average weight.

The tuberculin employed was Old Tuberculin (OT) supplied by the State Bacteriological Laboratory. The intradermal dosages were 1.0 and 0.1 mg. in a volume of 0.1 ml. of physiologic saline solution injected into the shaved abdominal skin.

Used as the nonspecific provoking agent was an *E. coli* filtrate (Seitz E.K.) prepared from six- to eight-day cultures in tryptic digest broth of *E. coli*, strain No. 652, isolated from a case of cystitis. The intravenous provocation of the tuberculous guinea pigs was carried out approximately seven weeks after infection, the dosage being 0.25 ml. of a dilution 1:20 or 1:40 of the coli filtrate, or 15 mg. of OT in volume of 0.25 ml. This corresponded approximately to one-half or one-quarter of the 50 per cent lethality end-point dose for these guinea pigs. The local provocation of the Mantoux sites was made by injecting into them 0.1 ml. of undiluted or diluted coli filtrate. As controls, tuberculin and coli filtrate were also introduced separately and at a distance.

The reactions at the tuberculin sites were recorded by mean lesion diameters of the erythematous swollen areas and hemorrhagic necrotic areas, respectively, twenty-four

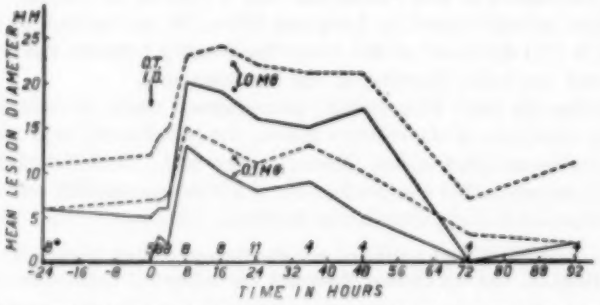

FIG. 1. Nonspecific hyperergic reactivity of tuberculin lesion to intravenous injection of coli filtrate at various time intervals after skin injection.

——— Hemorrhagic-necrotic areas
---------- Erythematous swollen areas
} Mean lesion diameters 24 hours after provocation with coli filtrate

* Number of animals in each group

hours after provocation or, in those cases provoked prior to Mantoux testing, twenty-four hours after the local tuberculin injection.

Biopsy specimens of tuberculin sites, taken under ether anesthesia, were fixed in 10 per cent $HgCl_2$, and sections were stained routinely with hematoxylin and eosin or van Giesen stain.

OBSERVATIONS

Development of Nonspecific Hyperergic Reactivity in Skin Tuberculin Lesions

In figure 1 are demonstrated the changes in the reactivity of tuberculin sites to nonspecific stimulation which occur as the specific inflammation proceeds.

> The ordinates represent the mean diameters of the skin lesions twenty-four hours after provocative intravenous injection (except of the first lesion, which was measured twenty-four hours after the tuberculin injection itself). The means refer to groups of 4 or 8 animals with two exceptions, as recorded in the figure. On the abscissa are plotted the times elapsed between the skin tuberculin injection and the intravenous coli filtrate provocation. The time of reading is thus constant in regard to the hour of provoking dosage, but varies, relative to the hour of tuberculin injection, as the interval between the two injections is extended. It should be noted, however, that with the dosages used the dimensions of the nonprovoked tuberculin lesions did not change appreciably between twenty-four and seventy-two hours after testing, as distinct from the intensity of the nonspecific reaction which varied greatly and decreased rapidly after the second day.

From the figure it will be seen that the hyperergic reactivity of the Mantoux site to nonspecific stimulus appeared sometime between the fourth and eighth hours and reached its maximum within eight hours after the tuberculin injection. This reactivity remained close to its top level for approximately forty-eight hours and disappeared on the third day. In a preliminary series of experiments, a slight reactivity was left a short while longer. The extent of the nonspecific lesions varied directly with the dosage of tuberculin.

Development of Nonspecific Hyperergic Reactivity and Infiltration of Polymorphonuclear Cells into Tuberculin Sites

The possibility that a Shwartzman-like mechanism may explain the nonspecific hyperergic reactions observed in tuberculosis has been discussed by several writers (1–5). A recent quantitative study by Gell and Hinde (13) has shown that in the tuberculin lesion an increase in both relative and absolute numbers of polymorphonuclear leukocytes occurs between four and twenty-two hours after the skin injection. This is in keeping with Laporte's (14) and Follis's (15) earlier observations as well as Wesslén's (16) more recent experience.

In view of evidence which has been presented to show that polymorphonuclear leukocytes may play an essential role in the preparation of the skin for the Shwartzman phenomenon (17), an experiment was designed to investigate whether the changes in reactivity of the tuberculin site to nonspecific provocation show any correlation to the infiltration by polymorphonuclear cells into the inflammatory area.

Guinea pigs, in groups of 2 or 4, were infected as described above, and were injected intracutaneously with 0.1 mg. of tuberculin at two symmetrical sites of their shaved abdominal skin. After specified intervals, one of the resulting lesions was removed for histologic examination, and immediately thereafter coli filtrate of the same estimated potency as in the first experiment was injected intravenously. The animals were observed twenty-four hours later for hyperergic reactions at the undisturbed tuberculin site.

A comparison of figure 2 with figure 1 reveals that the provocative effect of the coli filtrate in the dosage employed was somewhat weaker in this experiment than in the preceding one.

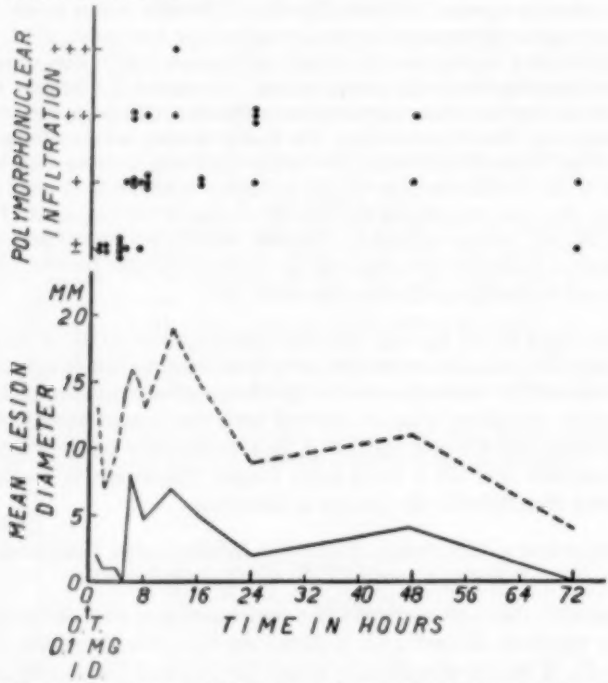

FIG. 2. Polymorphonuclear infiltration and nonspecific hyperergic reactivity of tuberculin lesion to intravenous injection of coli filtrate at various time intervals after skin injection.

——— Hemorrhagic-necrotic areas
---------- Erythematous swollen areas
} Mean lesion diameters twenty-four hours after provocation with coli filtrate

+++ predominant
++ marked
+ moderate
± slight or none
} Relative proportion of infiltrating polymorphonuclear leukocytes

● Each dot represents one animal

The histologic sections of the biopsied tuberculin lesions were surveyed for the relative proportion of polymorphonuclear cells, and the degree of nonspecific reactivity of the tuberculin site was demonstrated[4]. Six to seven hours after the tuberculin injection, when the first distinct increase in the proportion of polymorphonuclear leukocytes was reached, the first hemorrhagic reactions were also observed. As the relative proportion of these cells again decreased, the nonspecific reaction also diminished.

Reactivity of Dermal Tuberculin Lesions to Specific Intravenous Provocation

It is well known that tuberculin introduced into the circulation may cause a flare-up of a fading or already invisible local tuberculin reaction (18–20). It seemed of interest to study how a systemic injection of tuberculin would influence the developing local Mantoux reaction.

Apart from the substitution of tuberculin for the coli filtrate, the experimental conditions here did not differ from those of the preceding trials. In both cases the intravenous dosage was just below the lethal level. Each of the six guinea pig groups consisted of 4 animals.

It may be seen in figure 3 that no significant amplification of the specific inflammation resulted save a slight increment of a local reaction noted when the intravenous tuberculin provocation followed the Mantoux by eight hours.

The Effect of Coli Filtrate Injected into the Tuberculin Site at Various Intervals After the Tuberculin Injection

In figure 4 are shown the resulting inflammatory and hemorrhagic-necrotic reactions in tuberculin sites when undiluted coli filtrate was introduced locally (intradermally) into them at four-, eight-, sixteen-, or twenty-four-hour intervals after the tuberculin injection. The ordinates represent the mean diameters of the skin lesions twenty-four hours after the "provocative" injection of coli filtrate; the means refer to groups of 4 animals. The times elapsed between the intradermal tuberculin and the coli filtrate injections are plotted on the abscissa.

It will be seen that the filtrate modified the tuberculin inflammation markedly and, in the majority of animals, produced a more or less severe hemorrhagic-necrotic reaction. Maximum reactivity was noted in tuberculin sites eight and sixteen hours old. The same dosage of this coli filtrate, when injected into unprepared skin sites, displayed only an insignificantly weak inflammatory reaction. Lacking subsequent provocation by coli filtrate, the dosages of Old Tuberculin produced only typical erythematous indurations in which necrosis or hemorrhage was rare and minute.

The Comparative Effects of Coli Filtrate and Sterile Broth Injected in Various Concentrations into Neighboring Tuberculin Sites

Prompted by the observations of Freund and Hart (21) and Lichtenstein and Cilella (22), who found that tuberculous persons may give delayed skin reac-

[4] The writers wish to express their gratitude to Professor Nils Ringertz, M.D., who kindly performed these histologic examinations and whose estimates were made without prior knowledge of macroscopic appearance or expected result.

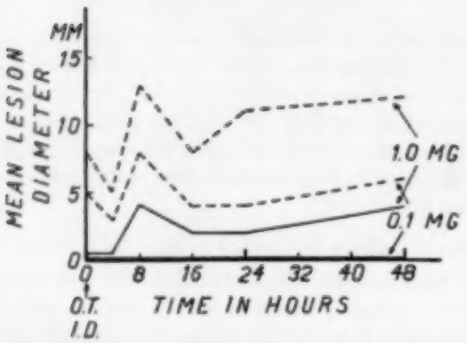

FIG. 3. Reactivity of tuberculin lesion to intravenous injection of tuberculin at various time intervals after skin injection

——— Hemorrhagic-necrotic areas
---------- Erythematous swollen areas

Mean lesion diameters twenty-four hours after provocation with Old Tuberculin (OT)

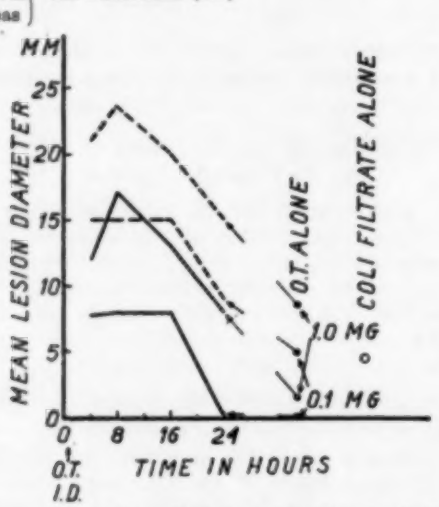

FIG. 4. The effect upon the tuberculin lesion of undiluted coli filtrate injected locally into the Mantoux site at various time intervals after the tuberculin injection.

——— Hemorrhagic-necrotic areas
---------- Erythematous swollen areas

Mean lesion diameters twenty-four hours after local injection of coli filtrate

○ Erythematous induration produced by coli filtrate alone

tions to sterile broth, a study was made to determine the comparative extent to which the tuberculin reactions were aggravated by subsequent injection of coli filtrate and sterile broth, respectively.

Thus, four groups of 4 guinea pigs apiece were prepared by symmetrical tuberculin injections into the shaved abdominal skin, using the same dosages as before.

In this manner, each pig received four OT injections, two of 1.0 mg. and two of 0.1 mg. in volumes of 0.1 ml. of sterile physiologic saline. After an interval of eight hours, 0.1 ml. of sterile broth was injected into one pair of tuberculin sites and 0.1 ml. of coli filtrate into the pair opposite. Advancing through the groups, the dilutions were 1:1, 1:4, 1:16, and 1:64, respectively. As controls, one group of pigs received an additional pair of tuberculin sites, into which was injected the same volume of sterile physiologic saline solution.

From figure 5 it may be seen that both coli filtrate and sterile broth aggravated the ensuing tuberculin reactions, but not to the same degree. The coli filtrate was the more active provoking agent, although in the undiluted preparations the difference between results was quite small.

COMMENT

The reactive state of the tuberculin site to nonspecific stimuli is transitory and stays at a high level only between the eighth and thirty-sixth to forty-eighth hours after the tuberculin injection. The degree of this nonspecific reactivity varies with the stage and severity of the tuberculin inflammation. Freund (7)

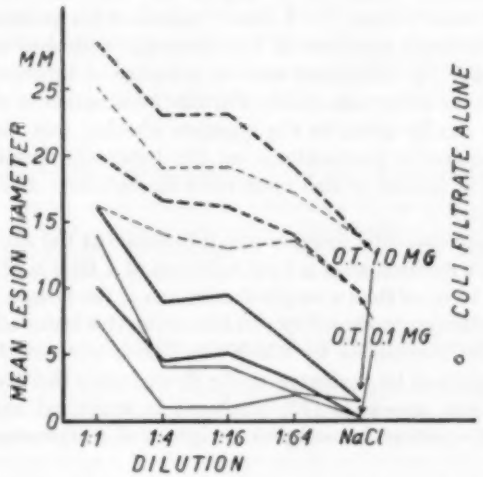

FIG. 5. The effect upon the tuberculin lesion of coli filtrate and sterile broth, respectively, injected into the Mantoux site in various concentrations eight hours after the tuberculin injection.

——— Hemorrhagic-necrotic areas
--------- Erythematous swollen areas
} Mean lesion diameters twenty-four hours after local injection of coli filtrate

——— Hemorrhagic-necrotic areas
--------- Erythematous swollen areas
} Mean lesion diameters twenty-four hours after local injection of sterile broth

○ Erythematous induration produced by coli filtrate alone

saw a similar correlation between the severity of tuberculin reaction and the extent of hemorrhage, but only when he studied the effect of several dilutions of tuberculin in the same guinea pigs and not when comparing the reactions of different animals.

Considering the essential role played by polymorphonuclear leukocytes in the preparation of the skin for the Shwartzman phenomenon (17), it is interesting to note that a similar relationship was demonstrated for the hyperergic reaction which occurs in a tuberculin site in response to nonspecific stimuli. This observation adds weight to the supposition that the basic mechanisms of both phenomena are similar.

It appears that intravenous tuberculin is a much less effective agent in producing local injury in the site of tuberculin inflammation than is nonspecific coli filtrate. In this connection, it may be interesting to recall that Old Tuberculin is equally poor an agent in eliciting the Shwartzman phenomenon (4). On the other hand, this observation contrasts directly with an earlier statement that in tuberculous guinea pigs the increase in systemic hyperergy is more pronounced to tuberculin than to nonspecific stimuli (2). The failure of provocative intravenous tuberculin injections to amplify the tuberculin inflammation is at variance with Freund's work (7). A closer analysis of his protocols reveals, however, that hemorrhagic reactions of the tuberculin test sites appeared only in those of his guinea pigs which had received a dosage of tuberculin large enough to produce a lethal tuberculin shock. Further information is required before a definite answer can be given to the question whether this dissimilar effect of specific and nonspecific provocations on the tuberculin lesions depends only upon quantitative factors or also upon more fundamental differences of underlying mechanisms.

The hemorrhagic-necrotic reaction which develops at the site of a moderately severe tuberculin reaction after a local injection of a filtrate of broth culture of *E. coli* seems to be more than a simple summation of the tuberculin inflammation plus the skin irritation produced by coli filtrate, as this latter effect by itself was insignificant. The mechanism by which the filtrate affected the tuberculin inflammation might well be analogous to the Shwartzman-like phenomenon which Black-Schaffer and associates (12) produced in sensitized animals through a mixed single injection of homologous antigen and a Shwartzman-preparatory toxin.

Under the same experimental conditions, however, sterile broth produced a similar, though somewhat weaker, reaction in the tuberculin site. It is true that broth does not ordinarily function as a Shwartzman-active agent (4), but this does not preclude the possibility that the special conditions of the present experiments may have released a Shwartzman-like mechanism ordinarily too inert to start under usual experimental circumstances. Alternately, it may also be considered possible that tuberculous animals can develop a nonspecific hyperergic reactivity to the broth medium itself, much as Freund and Hart (21) and, more recently, Lichtenstein and Cilella (22) have observed in certain human tuberculin reactors. Such an interpretation seems rather unlikely, however,

when it is recalled that the tuberculous animals of the present study did not develop nonspecific reactions to broth except following its direct injection into the tuberculin-prepared skin site.

SUMMARY

The dermal tuberculin inflammation of tuberculous guinea pigs reacts hyperergically to nonspecific stimuli. The degree of this reactivity varies with the stage of development and severity of the tuberculin reaction.

A direct relationship exists between degree of reactivity and infiltration by polymorphonuclear cells into the inflammatory area.

Specific intravenous provocation with tuberculin is less effective than nonspecific coli filtrate provocation in producing "focal" injury in the sites of tuberculin inflammation.

By direct injection of filtrate of *E. coli* broth culture into the sites of moderate tuberculin reactions of tuberculous guinea pigs, these reactions have been transformed into hemorrhagic-necrotic lesions. The possible mechanism of this phenomenon is compared with that of the single injection Shwartzman-like phenomenon described by Black-Schaffer and associates.

Sterile broth had an effect similar to coli filtrate, although in a lesser degree.

The observations made are consistent with the idea of a basic similarity between the underlying mechanisms of the nonspecific hyperergic reactions in tuberculosis and the Shwartzman phenomenon.

SUMARIO

Estudios de la Reactividad Hiperérgica Inespecífica en el Sitio de la Reacción a la Tuberculina

La inflamación dérmica producida por la tuberculina en los cobayos tuberculosos reacciona hiperérgicamente a estímulos inespecíficos. La intensidad de esa reactividad varía según el período de desarrollo y la gravedad de la reacción tuberculínica.

Existe una relación directa entre la intensidad de la reactividad y la infiltración por glóbulos polimorfonucleares en la zona de inflamación.

La provocación intravenosa específica con tuberculina es menos eficaz que la provovocación inespecífica con filtrados colibacilares para producir lesiones "focales" en los sitios de inflamación tuberculínica.

Mediante la inyección directa de filtrados de cultivos de *Escherichia coli* en caldo en los sitios de moderadas reacciones tuberculínicas en los cobayos tuberculosos, esas reacciones han sido transformadas en lesiones hemorrágico-necróticas. Compárase el posible mecanismo de ese fenómeno al fenómeno a la Shwartzman producido por una sola inyección que han descrito Black-Schaffer y colaboradores.

El caldo estéril ejerció un efecto semejante al del filtrado colibacilar, aunque menos intenso.

Las observaciones realizadas son compatibles con la idea de una semejanza básica entre los mecanismos subyacentes de las reacciones hiperérgicas inespecíficas en la tuberculosis y el fenómeno de Shwartzman.

RESUME

Etude sur la réactivité hyperergique non spécifique au niveau de la tuberculino-réaction

Chez le cobaye, la lésion inflammatoire du derme due à la tuberculine est le siège d'une réaction hyperergique aux stimulus non spécifiques. Le degré de cette réactivité varie avec le stade du développement et de la sévérité de la tuberculino-réaction.

Une relation directe existe entre le degré de la réactivité et l'infiltration de la zône inflammatoire par les polymorphonucléaires.

L'excitation spécifique par la tuberculine intraveineuse est moins efficace que l'excitation par filtrat de coli non spécifique pour produire la lésion "focale" au siége de la réaction inflammatoire tuberculinique.

L' injection directe de filtrat d'un bouillon de culture de *Escherichia coli* au niveau de tuberculino-réactions modérées chez le cobaye tuberculeux on a déterminé la transformation de ces réactions en lésions hémorragiques nécrotiques. Le mécanisme possible de ce phénomène est comparé à celui du phénomène pseudo-Shwartzman de l'injection unique, qui a été décrit par Black-Schaffer et collaborateurs.

Le bouillon stérile produisait une réaction semblable à celle du filtrat de coli, mais à un moindre degré.

Les observations faites sont en accord avec le concept d'une similarité basique entre les mécanismes fondamentaux des réactions hyperergiques en tuberculose et le phénomène de Shwartzman.

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THE CYTOLOGY OF THE TUBERCULIN REACTION IN SKIN WINDOWS IN MAN[1,2]

JOHN W. REBUCK AND J. LEWIS YATES

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INTRODUCTION

The leukocytic cycles in acute inflammation in experimental animals were described originally by Metchnikoff (1, 2, 3). The basic sequences consisted of an early migration of phagocytic granulocytes reinforcing available tissue macrophages. These phagocytes were supplemented by migrating lymphocytes and blood mononuclears (monocytes) which hypertrophied to form additional macrophages. Recently the present writers have reviewed the experimental work reaffirming these basic cellular concepts by advanced techniques (4, 5) and have confirmed this description of the acute inflammatory cycle in skin windows in man.

The present communication is a preliminary report of a technique which will permit tissue examinations to be performed repeatedly in the same subject in different stages of the tuberculin reaction in the same lesion. Furthermore, subsequent lesions can be similarly studied in the same person.

MATERIALS AND METHODS

Physician volunteers with negative or positive cutaneous reactions to tuberculin were used in the preliminary investigations. The subjects who presented local hypersensitive reactions to tuberculin were free of clinical evidence of active tuberculosis.

Two lots of Koch's Old Tuberculin (OT) were used. One lot was prepared by the Minnesota State Health Laboratories and the other by the Parke, Davis Laboratories. In addition, purified protein derivative (PPD)[3] in general use at The Henry Ford Hospital was utilized.

Experimental lesions were prepared by scraping the epithelium from a small area of the volar skin surface of the forearm. The tuberculin preparation to be studied was applied topically (0.05 cc.) to the denuded surface; the material was not injected. The lesion was covered with a sterile coverslip, overlaid with a square of sterile cardboard, and the entire area was in turn surmounted by surgical adhesive tape. Cells of the earliest exudative sample migrated to the undersurface of the coverslip within the first hour. The time of epidermal removal marked the time of beginning inflammation. When the first cellular migrations had been effected, the coverslip was removed, air-dried, and stained like a blood smear with May-Grünwald-Giemsa dyes. At the time the first coverslip was removed, another coverslip, held in readiness, was placed similarly over the same lesion and the process was repeated at timed intervals throughout the first, second, and occasionally the third day of the inflammatory reaction.

Five lesions in subjects with negative cutaneous reactions were studied: one

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[1] From the Department of Laboratories and the Cardio-Respiratory Division, The Henry Ford Hospital, Detroit 2, Michigan; and the Department of Anatomy, the University of Minnesota, Minneapolis 14, Minnesota.

[2] Presented before the Medical Session, as part of the session on *Research*, at the annual meeting of the National Tuberculosis Association, Los Angeles, California, May 21, 1953.

[3] From Parke, Davis Laboratories.

after initial application of 0.05 cc. of 1:10,000 OT at 1.75, 3.75, 6, 9, 10, 12, 14, 24, 31, and 47.5 hours; one after application of 0.05 cc. of 1:1,000 OT at 3, 6, 9, 12, 14, 21, and 24 hours; and three, after application of 0.05 cc. of first-strength PPD at 1.75, 2.25, 3.75, 5.25, 6, 7.25, 9, 10, 12, 14, 24, 31, and 47.75 hours.

Nine lesions were similarly studied in the tuberculin-positive subjects described above: one after application of 0.05 cc. of 1:10,000 OT at 2, 3.75, 6, 8, 10, 12, 14, 24, 30, and 48 hours; three after topical application of 0.05 cc. of 1:1,000 OT at 4, 5.5, 8, 9, 12, 13.5, 14, 14.5, 16.5, 18, 20, 22, 24, 28, 30, 39, 51, and 63 hours; and five after application of 0.05 cc. of first-strength PPD at 2, 3.75, 5.6, 8, 8.5, 10, 12, 14, 24, 25, 30, and 48 hours of inflammation.

The leukocytic cycles observed in the local reactions to tuberculin were in turn compared with the leukocytic cycles observed in local reactions to diphtheria toxoid (4, 5, 6) in normal subjects and in patients suffering from Boeck's sarcoid (6). Such leukocytic responses were further compared with the monocytic reactions evoked in local inflammatory sites in patients (7) and animals (8) with high blood monocytoses.

The lesions in preparation and study were almost devoid of pain and elicited no subjective complaints. After study they healed promptly under a small sterile dressing.

RESULTS

Tuberculin-Negative Subjects

The leukocytic sequences of the local inflammatory response to tuberculin in tuberculin-negative subjects are presented in the following composite descriptions.

In general, the leukocytic cycles corresponded closely to the responses de-

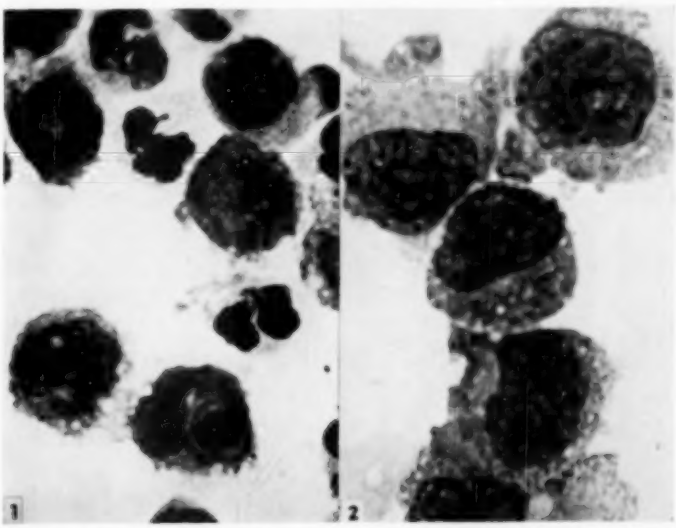

FIG. 1. Normal control. Twelve hours' inflammation in man after diphtheria toxoid. Lymphocytes and degenerating neutrophils. ×1800.

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FIG. 2. Normal control. Twenty hours' inflammation in same lesion as figure 1. Note predominance of macrophages. ×1800.

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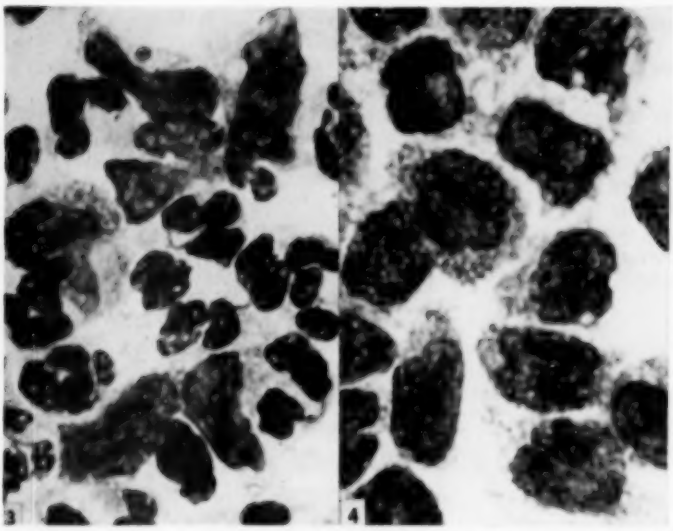

FIG. 3. Tuberculin-negative subject. Nine hours' inflammation in man after Old Tuberculin. Lymphocytes and neutrophils. ×1800.

FIG. 4. Same lesion as figure 3 but now twelve hours' inflammation in man after CT. Numerous lymphocytes show slight hypertrophy. ×1800.

scribed (4, 5) for other nonpyogenic antigenic stimuli in control lesions (figures 1 and 2). The 2- to 8-hour responses were characterized by a pronounced migration of neutrophilic leukocytes with minor participation by the local tissue macrophages.

Although minimal lymphocytic participation had been observed earlier, at 8 to 12 hours the lymphocytes appeared in the lesions in increasing numbers (figure 3). At 12 to 14 hours the neutrophils shed the cytoplasm peripheral to their nuclear lobes, became pyknotic, and presented other degenerative changes. In this same period, the lymphocytes underwent moderate cytoplasmic hypertrophy (figure 4).

Phagocytic ability, when expressed, was proportional to an increasing cytoplasmic mass (9). Nuclear changes were shown by a breaking up of the coarse, chromatin masses into smaller units. The normal indentation of the lymphocyte, usually obscured in the blood, was occasionally accentuated (10). From 14 to 24 hours (figure 5), the progressive changes in the hypertrophying lymphocytes eventuated in assumption of structural characteristics in both nucleus and cytoplasm which rendered them indistinguishable, by these techniques, from the relatively few tissue macrophages originally participating in the response.

From 24 to 48 hours, the presence of numerous macrophages in the local inflammatory site was concurrent with a clearing of the experimental lesions.

The times of appearance of the successive leukocytic participants and their transformational activities were reproduced in the various lesions within sur-

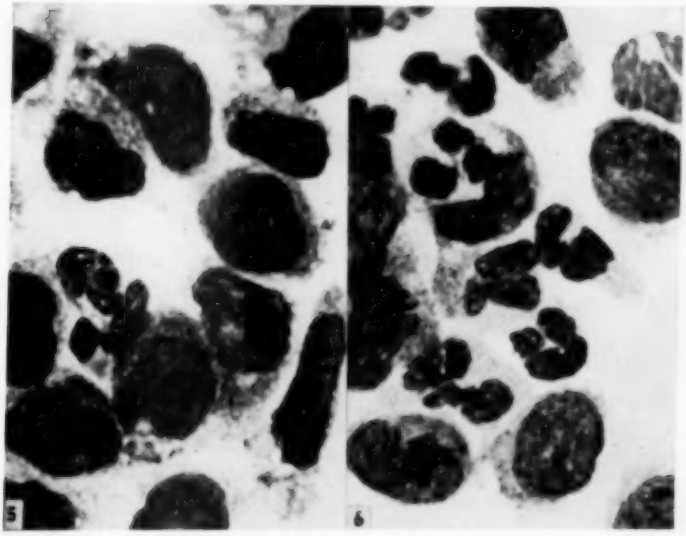

FIG. 5. Same lesion as figure 3 but now fourteen hours' inflammation in man after OT. Numerous lymphocytes show hypertrophy. ×1800.

FIG. 6. Tuberculin-negative subject. Twelve hours' inflammation in man after purified protein derivative (PPD). Lymphocytes and neutrophils. ×1800.

prisingly narrow limits. The peak of lymphocyte participation, as lymphocytes, at the important 12-hour stage varied from moderate participation (figure 6) in some instances to complete field dominance (figure 4) in others. Mitoses were not observed in the exudative cells.

Tuberculin-Positive Subjects

In contrast to the leukocytic sequences described above for the local responses to topically applied tuberculin in experimental lesions in tuberculin-negative subjects, the exudative response in lesions in persons who presented positive cutaneous reactions to tuberculin showed several marked deviations.

These deviations appeared as three types of altered leukocytic cycles. In the first type of altered cycle, represented by three of the lesions, the initial neutrophilic response gave way at the 8- to 9-hour period to a precocious and intense lymphocytic infiltration (figure 7). Lymphocytic predominance, together with slightly hypertrophied lymphocytes, continued through the 12-hour stage in two of these lesions, only to be supplanted at the 24-hour stage by a second migration of large numbers of neutrophils (figure 8). In the third lesion of this group, the precocious lymphocytic infiltrate had already been replaced by a pyogenic response at the 12-hour stage. When the first type of altered response was followed through the third day, the more normal mononuclear cycles of lymphocytes, hypertrophied lymphocytes, and macrophages were not initiated until 39 to 51 hours and eventuated in large macrophages and giant cells at 63 hours.

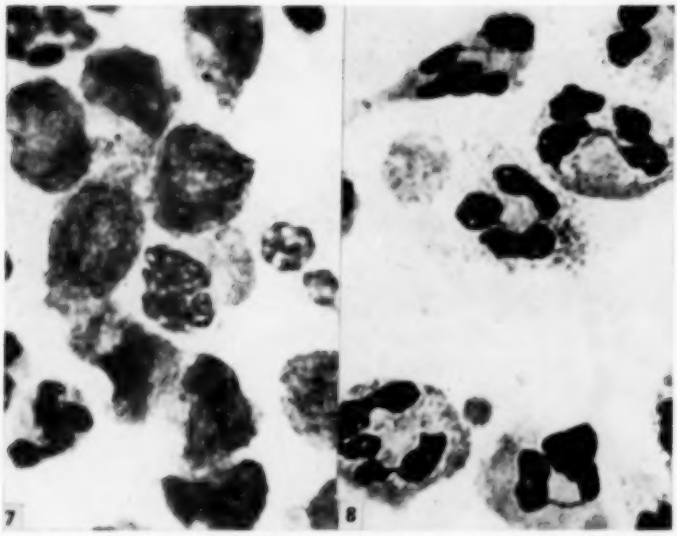

FIG. 7. Tuberculin-positive subject. Nine hours' inflammation in man after OT. Note precocious response of lymphocytes. ×1800.

FIG. 8. Same lesion as figure 7 but now twenty-four hours' inflammation in man after OT. Note defective exudate with excess neutrophils at this stage. Compare with figure 2. ×1800.

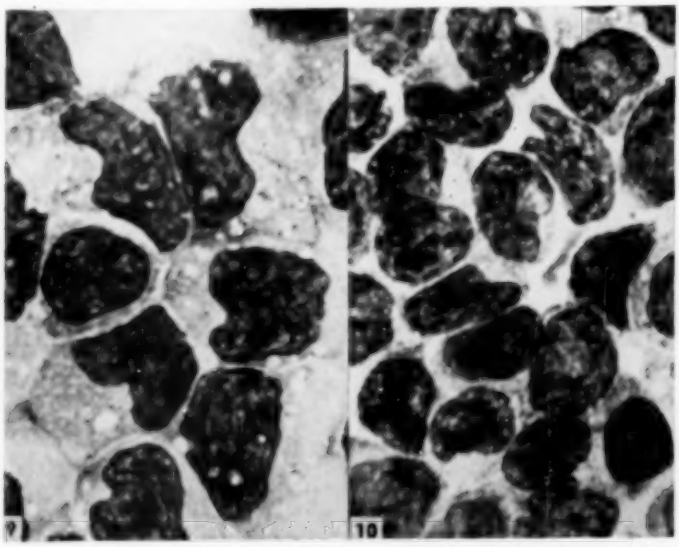

FIG. 9. Tuberculin-positive subject. Eight hours' inflammation in man after PPD. Note excess of macrophage response for this stage. Compare with figure 3. ×1800.

FIG. 10. Tuberculin-positive subject. Twenty-four hours' inflammation in man after PPD. Note compact masses of lymphocytes. Note retardation of response depicted in figure 2. ×1800.

In the second type of altered sequences, the initial neutrophilic response was replaced by the precocious but not necessarily predominant appearance of large numbers of macrophages (figure 9) far in excess of the small number of tissue macrophages ordinarily present at this time in control lesions. At 12 hours this second type of altered cycle presented large numbers of macrophages and neutrophils with peak lymphocytic participation retarded until the 24-hour stage. At 24 hours in this group, represented by two lesions, the predominantly mononuclear exudate presented a highly pleomorphic response of lymphocytes, hypertrophied lymphocytes, and macrophages, a picture commonly present at 12 to 14 hours in controls (*see* figures 1, 4, and 6).

In the third type of altered cyclic responses in the four remaining lesions to be described, the initial neutrophilic response was accentuated at the 8-hour stage and reinforced by macrophages at the 12-hour stage. The customary lymphocytes and hypertrophied lymphocytes at the 12- to 14-hour stages were again depressed. At 24 hours (figure 10), a retarded, pleomorphic response of lymphocytes, hypertrophied lymphocytes, and macrophages resulted. In many areas the lymphocyte was still the predominant cell type. Although mitotic figures were not observed in any of the exudative cells, bi- and tri-nucleated macrophages were common at the 24-hour stage.

The blood monocytes contributed to macrophage production in the inflammatory responses in a degree roughly proportional to the monocyte content of the

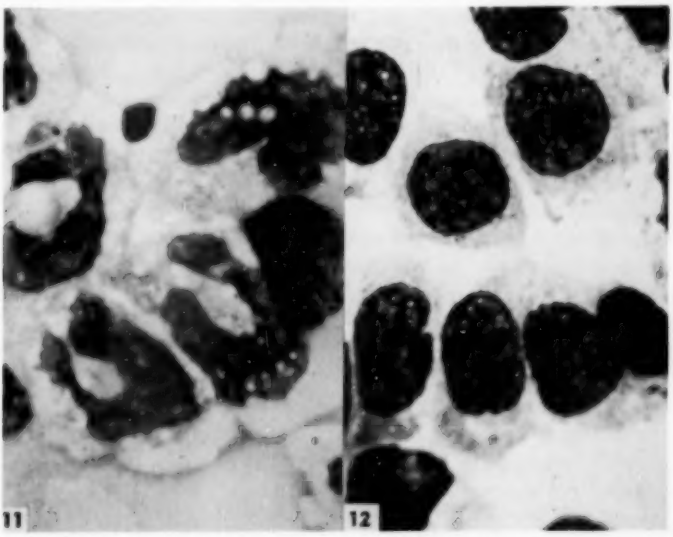

FIG. 11. Subject with high blood monocytosis. Inflammation in man at 12.75 hours. Note monocytes. Compare with figures 4 and 6. ×1800.

FIG. 12. Case of Boeck's sarcoid. Peculiar massed macrophage response after corticotropin. Inflammation in man after 5.75 hours. In normal controls, neutrophilic leukocytes predominate at this stage. ×2000.

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peripheral blood. In a patient with a high peripheral monocytosis, whose leukocytic inflammatory cycles will be the subject of a future communication, the monocytes migrated in large numbers in response to a nonpyogenic antigen. At 12 hours of inflammation (figure 11), the numerous monocytes presented accentuation of their horseshoe-shaped nuclear configuration and cytoplasmic hypertrophy in excess of their original large cytoplasmic mass in the bloodstream. When the cells in figure 11 are contrasted with the other photomicrographs of the leukocytic responses to tuberculin (*see* figures 3, 4, 5, 6, 7, 9, and 10), it will be apparent that the lymphocytes, hypertrophied lymphocytes, and macrophages of the latter responses are structurally distinguishable from monocytes and hypertrophied monocytes. That blood monocytes participate in local defensive processes in proportion to their numbers in the blood has been demonstrated previously in experimental animals by several groups of investigators (8, 11, 12,).

The large masses of closely packed macrophages which unprecedentedly composed most of the exudative cells at 5.75 hours of inflammation in responses to either tuberculin or diphtheria toxoid in a patient suffering from Boeck's sarcoid (6) (figure 12) should be compared with the similar but less striking precocious macrophage response depicted in figure 9 at 8 hours of inflammation.

DISCUSSION

Neutrophilic leukocytes were predominant in the early hours of the hypersensitive reactions to tuberculin in the above-described experimental lesions in man. This finding is in general agreement with previously reported observations of Laporte (13) and Follis (14), but differs from the earlier experience of Dienes and Mallory (15), who described mononuclear predominance at the site of the tuberculin reaction from the second hour on. Rich (16) has indicated the exaggeration of the neutrophilic migrations present in the hypersensitive reaction far exceeding the neutrophilic response to comparable stimuli in the normal body. Preliminary investigations in the present study substantiate this concept. In all three of the altered successions of leukocytic events, accentuation of the neutrophilic response played an important, although perhaps compensatory, role. In the first type, precocious lymphocytic predominance gave way to undue neutrophilic participation particularly well marked at the 24-hour stage, a period of macrophage predominance in control lesions. In the second and third types of altered sequence, neutrophilic predominance throughout most of the first day appeared to be on the basis of lymphocytic deficit.

The succeeding mononuclear responses have been complicated by the tendency to report such responses to the tubercle bacillus or its constitutents in normal and hypersensitive tissues as if they were of common origin and structure.

Reference to figures 3, 4, 5, 9, 11, and 12 will indicate that mononuclear responses in acute inflammation in man may be composed of lymphocytes, monocytes[4], hematogenous macrophages (the transformational products of lympho-

[4] Monocyte in this paper is used in the contemporary hematologic sense of the transitional leukocyte and large mononuclear of Ehrlich and not as synonymous with histiocytes, macrophages, and large phagocytic mononuclears of inflammation.

cytes and monocytes), or tissue macrophages (histiocytes, clasmatocytes, or reticulum cells), or any mixture of these cell types. Almost all workers agree that monocytes can form macrophages (17). In his first work (1) on macrophage formation in mammals and later in his monograph (3), Metchnikoff stressed the importance of the lymphocyte as the hematogenous source of macrophages. It is pertinent that this first work (1) consisted of studies on the cellular responses to pulmonary tuberculosis in rabbits and marmots. Many careful workers at the time of Metchnikoff and even to this day have denied that macrophages can develop from lymphocytes. There has been continued confirmation of Metchnikoff's demonstration of this lymphocytic function, however, up to the present decade by Maximow (18), Downey (19), Bloom (20), Taliaferro and Mulligan (21), Kolouch (22), and many others. During the past decade or so the concept that lymphocytes can develop into macrophages has been reaffirmed through utilization of modern technique in investigations of the central nervous system (23, 24, 25), in connective tissue spreads stained like bone marrows (26, 27), in motion pictures (28) and dry films (29) of tissue cultures, in continuous observations of living lymphocytes (30), and in skin windows in man (4, 5, 6, 7).

The present preliminary investigations of the cutaneous hypersensitive reactions to tuberculin should be considered against the background of these basic cellular concepts. The results confirm the previously reported altered events of exaggerated polymorphonuclear leukocytic participation and late but accentuated mononuclear responses in their reaction. The results further suggest that interruption of lymphocytic cycles at the time of normal or even precocious lymphocytic performance leads to defective and ultimately retarded exudative responses to such stimuli.

Monocyte participation did not approach in magnitude that found in lesions in controls which possessed high peripheral blood monocytoses, nor was the early macrophage excess of the sarcoid lesions as marked in the tuberculin-reactive preparations.

SUMMARY

A preliminary report of a technique which will permit repeated sampling at timed intervals of the exudative cells from a single lesion responding to tuberculin in man has been presented. Subsequent lesions can be similarly studied in the same person. The preparations can be stained as blood smears. The successions of leukocytic migrations and participation were studied in 5 lesions in tuberculin-negative and in 9 lesions in tuberculin-positive volunteers.

The cellular responses in lesions in subjects with negative skin tests corresponded to similar responses to other control, nonpyogenic antigens. The cellular exudate consisted of tissue macrophages and neutrophils for 8 hours, appearance of lymphocytes at 8 to 12 hours, followed at 12 to 14 hours by hypertrophying lymphocytes, and at 16 to 48 hours by the resultant macrophage cell mass.

Marked alterations in the leukocytic sequences in the cutaneous hypersensitive reaction were observed in lesions in subjects with positive skin tests. These alterations resulted in defective and retarded mononuclear responses which were

due to depression of lymphocytic participation at various stages in the inflammatory responses. In two groups, outright lymphocytic deficit led to macrophage or neutrophilic accentuation for 24 hours, covering the period of normal lymphocytic participation. In a third group, early lymphocytic migration was replaced by a second migration of neutrophils supplanting macrophage predominance at 24 hours.

The leukocytic cycles established in both groups were compared with the bizarre cycles established in a patient with a high blood monocytosis and in patients with Boeck's sarcoid. At no time did monocyte participation approach in magnitude that found in lesions in controls who possessed high, peripheral monocytoses; nor was the early macrophage response of the sarcoid lesions as marked in the cutaneous hypersensitive reactions to tuberculin.

SUMARIO

La Citología de la Reacción Tuberculínica en las Ventanillas Cutáneas en el Hombre

Preséntanse aquí reseña preliminar de una técnica que permitirá obtener muestras repetidas a plazos fijos de las células exudativas procedentes de una lesión aislada formada como reacción a la tuberculina en el hombre. Las lesiones subsiguientes pueden ser estudiadas en forma semejante en la misma persona. Las preparaciones pueden teñirse lo mismo que frotes sanguíneos. Las sucesiones de migraciones y participación leucocíticas fueron estudiadas en 5 lesiones en voluntarios tuberculino-negativos y en 9 en voluntarios tuberculino-positivos.

Las respuestas celulares en las lesiones de los sujetos con cutirreacciones negativas correspondieron a las respuestas similares a otros antígenos no piógenos que sirvieron de testigos. El exudado celular comprendió macrófagos y neutrófilos histiónicos durante ocho horas, aparición de linfocitos al cabo de ocho a doce a horas, seguida a las doce a catorce horas de linfocitos hipertrofiantes y a las dieciséis a cuarenta y ocho horas de la resultante masa macrofagocítica.

En las lesiones de los sujetos con cutirreacciones positivas, observáronse notables alteraciones en las series de fenómenos leucocíticos de la cutirreacción hipersensible. Dieron por resultado esas alteraciones respuestas mononucleares defectuosas y retardadas debidas a la depresión de la participación linfocítica en varios períodos de las respuestas inflamatorias. En dos grupos, el neto déficit linfocítico condujo a acentuación macrofágica o neutrófila durante veinticuatro horas, abarcando esto el período de participación linfocítica normal. En un tercer grupo, la migración linfocítica temprana fué reemplazada por otra migración de neutrófilos, que suplantó a las veinticuatro horas el predominio de macrófagos.

Los ciclos leucocíticos establecidos en ambos grupos fueron comparados con los ciclos peculiares establecidos en un enfermo que tenía hipermonocitosis y en enfermos con el sarcoide de Boeck. En ninguna ocasión se aproximó en magnitud la participación monocítica a a la observada en las lesiones del testigo que tenía hipermonocitosis; ni tampoco fué la temprana respuesta macrofágica de las lesiones sarcoideas tan acentuada como en las cutirreacciones hipersensibles a la tuberculina.

RESUME

La cytologie de la réaction à la tuberculine étudiée dans les "skin windows" (fenêtres cutanées) chez l'homme

Présentation d'un rapport préliminaire sur une technique qui permettra, chez l'homme, la répétition d'examens périodiques au niveau d'une seule lésion de réaction à la tuberculine. Les altérations ultérieures peuvent être observées de façon semblable chez un même sujet. La coloration des préparation est faite comme celle des lames de sang. Les migrations succes-

sives et la participation des leucocytes furent étudiées dans 5 lésions chez des volontaires ayant une réaction négative à la tuberculine et dans 9 lésions chez des volontaires ayant une réaction positive.

Les réactions cellulaires des lésions chez les sujets à réaction cutanée négative correspondent à celles des lésions déterminées par des antigènes non pyogènes. L'exudat cellulaire consistait en macrophages et neutrophiles pendant une période de huit heures; les lymphocytes apparaissaient entre la huitième et la douzième heure; ensuite, entre la douzième et la quatorzième heure survenait l'hypertrophie lymphocytaire et enfin, entre la seizième et la quarante-huitième heure, la masse, cellulaire des macrophages.

Chez les sujets à réaction cutanée positive l'ordre de succession leucocytaire était nettement altéré dans les réactions d'hypersensibilité cutanée. L'altération se traduisait par un retard de la réponse mononucléaire par réduction de la participation lymphocytaire à divers stades de la réaction inflammatoire. Dans deux groupes, un véritable déficit lymphocytaire a conduit à l'accentuation d'une phase macrophage ou neutrophile durant vingt-quatre heures, correspondant à la phase de participation leucocytaire normale. Dans un troisième groupe, une migration leucocytaire précoce fut remplacée par une seconde migration de neutrophiles au lieu de la prédominance macrophage de la vingt-quatrième heure.

Les cycles leucocytaires déterminés dans les deux groupes furent comparés chez un malade ayant une mononucléose élevée et chez des malades atteints de sarcoïdose de Boeck. A aucun moment la participation monocytaire n'a approché l'importance de celle des lésions chez les sujets témoins présentant une monocytose périphérique élevée; de même, la réaction macrophage précoce des sarcoïdes n'était pas aussi marquée que celle des réactions cutanées allergiques à la tuberculine.

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TUBERCULIN-HISTOPLASMIN CONVERSION RATES IN KANSAS CITY AS AN INDICATION OF THE PREVALENCE OF INFECTION—PRELIMINARY REPORT[1, 2]

I. Tuberculin Studies

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(Received for publication May 19, 1953)

INTRODUCTION

The increasing costs and diminishing returns of mass roentgenographic surveys make it necessary for tuberculosis associations, official health agencies, and others concerned with tuberculosis control to constantly re-evaluate their tools and determine the advantages and limitations of the various available methods. This is particularly important in the central United States, where the prevalence of histoplasmin sensitivity is so high. Since differential roentgenographic diagnosis of tuberculosis and fungus infection of the lungs is so difficult, mass survey findings frequently lead to confusion among both patients and private physicians.

The present paper reports the results of studies among schoolchildren in Kansas City, Missouri, employing as tools tuberculin and histoplasmin skin tests, roentgenograms, and family studies.

MATERIAL AND METHODS

Tuberculin and histoplasmin skin tests are performed annually in the public school kindergarten and first-grade children by the Kansas City Tuberculosis Society. Similar tests are performed in parochial schools by the City Health Department. Nonreactors in the kindergarten group are retested the following year. The total number of subjects tested at approximately yearly intervals ranges from 2,900 to 3,900 children.

In addition to the above, a special study is being conducted in seven parochial schools. The purpose is to follow a fairly large group of skin-test-negative children by frequent retesting with tuberculin and histoplasmin to determine seasonal changes in infection rates, particularly to histoplasmin. This study was started in the 1949–1950 school year and has continued to the present. The initial group consisted of children in the kindergarten and the first three grades. The nonreactors of this group have been retested at intervals of six months for four years. Each year the incoming kindergarten class is added to the original group so that the number of children included in this study has increased from 700 to 1,200.

All positive reactors to either antigen receive a 70 mm. or a 4- by 10-inch chest roentgenogram and, if any abnormality is noted, a standard-sized roentgenogram is made.

The families of all tuberculin-positive children and the "converters" to the tuberculin test are visited and urged to have miniature films. If the small film shows any abnormality, a standard-sized chest roentgenogram is obtained. If abnormal findings are noted on stand-

[1] From the Kansas City Tuberculosis Society and the Tuberculosis Division, Kansas City, Missouri, Health Department.

[2] Presented before the Medical Session, as part of the session on *Nontuberculous Diseases of the Chest*, at the annual meeting of the National Tuberculosis Association, Los Angeles, California, May 19, 1953.

ard-sized films, either child or adult, they are referred to their family physician or to the Health Department Tuberculosis Clinic for clinical evaluation. Based on this evaluation, the case of the person involved is classified as *Active*, *Inactive*, or *Pending* (if examination is incomplete). The active tuberculosis cases are further classified as *New* or *Known*, based on the Health Department Tuberculosis Register.

The tuberculin employed on these surveys was commercial purified protein derivative (PPD). The dose was 0.0001 mg. All tests were intracutaneous and were read at forty-eight hours. A positive reaction was one in which the induration measured 5 mm. or more.

For the purposes of the present report, the analysis has been limited to findings among kindergarten and first-grade children except for the results of the follow-up of histoplasmin "converters" in which 9 older children are included whose skin tests changed from negative to positive under observation.

RESULTS

In figure 1 are shown the tuberculin rates by different sections of the city among kindergarten children in Kansas City. It is obvious that certain school

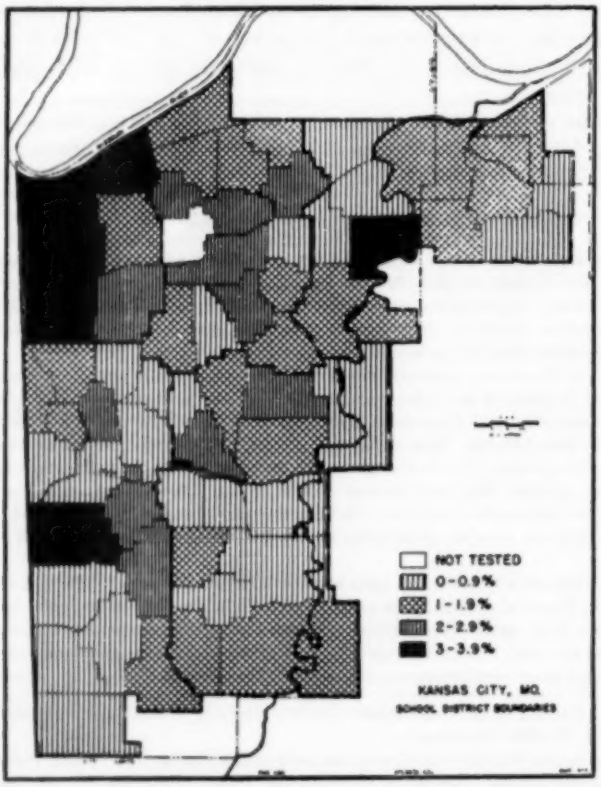

FIG. 1. Tuberculin sensitivity among public school kindergarten children, 1947–1952, in Kansas City, Missouri.

TABLE 1

COMPARISON OF TUBERCULIN SENSITIVITY WITH TUBERCULOSIS ANNUAL DEATH RATE BY SECTIONS OF KANSAS CITY, MISSOURI

	SECTIONS OF THE CITY				
	Northeast	North Central	North-west	South-east	South-west
Tuberculin sensitivity in kindergarten (public schools), 1947–1952	1.3%	1.7%	3.1%	1.2%	1.2%
Tuberculosis annual death rate per 100,000, 1949–1951	13.5	15.9	60.6	7.9	8.9

districts have excessively high rates, this being particularly true in the northwest. Other areas appear to have low rates, especially in the southern part of the city, except for one district.

In table 1 are shown the comparisons of the rate of tuberculin sensitivity in kindergarten children with the tuberculosis death rate by sections of Kansas City. The parallel between the tuberculosis death rates and the tuberculin sensitivity rates is evident. Thus, the area in the northwest section of the city, which has the highest tuberculosis death rate (60.6 per 100,000), also has the highest prevalence of tuberculin sensitivity (3.1 per cent). The two areas with the lowest rates, the southeast and southwest sections, show the lowest rate to tuberculin sensitivity (1.2 per cent), which is approximately one-third of that shown in the northwest section.

Previous data on tuberculin sensitivity collected in Kansas City during 1945–1946 have been reported (1). The dots in figure 2 show the percentage of positive reactors for the various age groups. The line drawn on the figure represents a theoretical annual conversion rate calculated for this group by the Ross method (2). The calculated rate of 0.58 per cent per year would account for the observed rate of tuberculin reactors among these children up to age 18. It is quite evident from viewing the figure that the theoretical annual conversion rate of approximately 0.6 per cent per year offers a reasonable explanation of what one might expect to occur in the Kansas City children with regard to tuberculosis infection.

In table 2 is shown the annual conversion rate to tuberculin based on repeated tests. The results are shown by sections of the city. It is noted that in the year beginning September, 1950, 10 "converters" of 1,251 previously negative children were observed, a rate of 0.8 per cent. In the year beginning September, 1951, 1,170 previously negative children were retested, 9 (0.8 per cent) of whom had converted to the tuberculin-positive state. It is of interest to point out that the rates were the same for the two years, indicating the veracity of the observed infection rate. This rate is somewhat higher than the theoretical rate of approximately 0.6 per cent per year as was shown in figure 2.

In table 3 are shown the results of the follow-up of the tuberculin-positive children and their contacts. The table is divided into two parts, the first being the follow-up of the contacts of 24 children who converted to the tuberculin test. These children were approximately six years of age. Of 57 adults in the

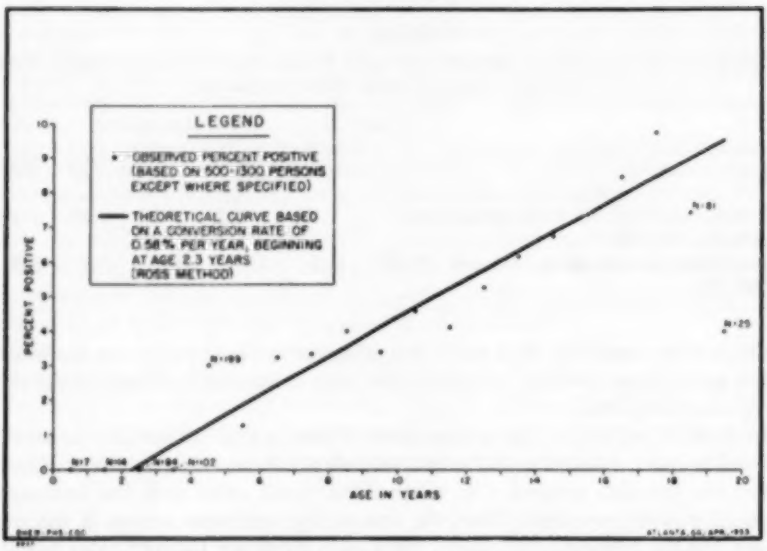
LEGEND
OBSERVED PERCENT POSITIVE (BASED ON 500-1300 PERSONS EXCEPT WHERE SPECIFIED)
THEORETICAL CURVE BASED ON A CONVERSION RATE OF 0.58% PER YEAR, BEGINNING AT AGE 2.3 YEARS (ROSS METHOD)
PERCENT POSITIVE
AGE IN YEARS

FIG. 2. Observed and theoretical tuberculin sensitivity rates by age among Kansas City, Missouri, children (1945 survey).

TABLE 2

"CONVERTERS" TO THE TUBERCULIN TEST

Number and Percentage of Children Whose Tuberculin Tests Were Negative in the Kindergarten and Positive in the First Grade One Year Later. Two Years of Observation.

SCHOOL YEAR BEGINNING		SECTIONS OF THE CITY					TOTAL
		North-east	North Central	North-west	South-east	South-west	
1949	Number of negatives re-tested	228	303	120	316	284	1,251
	Number of "converters"	3	3	1	0	3	10
	Per cent	1.3	1.0	0.8	0.0	1.1	0.8
1950	Number of negatives re-tested	191	267	97	291	324	1,170
	Number of "converters"	5	2	1	1	0	9
	Per cent	2.6	0.7	1.0	0.3	0.0	0.8
Combined years	Number of negatives re-tested after one year	419	570	217	607	608	2,421
	Number of "converters"	8	5	2	1	3	19
	Per cent	1.9	0.9	0.9	0.2	0.5	0.8

households of these tuberculin "converters," 54 were followed and 3 new active cases (5.6 per cent) were found. In addition, one known case was found in a contact, making a total of more than 7 per cent of cases of active tuberculosis among

TABLE 3

FOLLOW-UP STUDIES OF 1,396 CONTACTS OF 319 TUBERCULIN-POSITIVE CHILDREN IN PUBLIC AND PAROCHIAL SCHOOLS (1948–1952) IN KANSAS CITY, MISSOURI

TUBERCULIN STATUS OF INDEX CHILD	NUMBER AND DESCRIPTION OF CONTACTS		CASES OF TUBERCULOSIS									
			Active				Inactive		Pending*		Total	
			New		Known							
			Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
24 "Converters"	Household	54 Adults	3	5.6	1	1.9					4	7.4
		31 Children									0	0.0
	Nonhousehold	32 Adults			1	3.1			3	9.4	4	12.5
		11 Children									0	0.0
295 Positive on first test	Household	609 Adults	9	1.5	27	4.4	4	0.7	11	1.8	51	8.4
		328 Children	6	1.8	2	0.6	5	1.5			13	4.0
	Nonhousehold	273 Adults	1	0.4	22	8.1	2	0.7	4	1.5	29	10.6
		58 Children			1	1.7					1	1.7

* Suspicious of tuberculosis, but final status not determined.

the 54 adults who were contacts with the "converters." No tuberculosis was found among the 31 children who were household contacts with these "converters." Two children were not followed. Among the 32 adult nonhousehold contacts, one known case of tuberculosis and 3 cases classified as still pending were found.

In the second part of the table are shown the results of the follow-up of the contacts of 295 kindergarten and first-grade children who were tuberculin positive on the first test. A total of 609 adult household contacts were followed, of whom 9 (1.5 per cent) were found to be new active cases of tuberculosis. An additional 27 persons (4.4 per cent) were found to have known active tuberculosis. Eleven cases were designated as pending, and 4 cases of inactive tuberculosis were also found. Among these 609 adults, therefore, 51 (8.4 per cent) definite or suspected cases of tuberculosis were encountered. An additional 68 adults could not be followed.

Among the 328 children who were household contacts of these 295 tuberculin-positive children, 6 (1.8 per cent) new cases of active tuberculosis were found. Included with these 6 cases are 3 among the tuberculin-positive children themselves who served as the index cases. These 3 children were diagnosed as having active tuberculosis, previously unknown. Two other children were found who represented known cases of tuberculosis. Five cases of inactive tuberculosis were found. Twenty-two children could not be followed. Among the nonhousehold contacts of these 295 children, there were 273 adults among whom one new active case of tuberculosis was found, 22 known active cases, 4 cases still pending, and 2 inactive cases. A total of 29 (10.6 per cent) of these adults showed some evidence of tuberculosis. Among 58 nonhousehold contact children investigated, one known case of tuberculosis was found.

It is of considerable interest to point out that, among a total of 1,022 adults and children who were household contacts of tuberculin-positive children in the kindergarten or first grade, approximately 1.8 per cent were found to represent previously unknown active cases of tuberculosis. In addition, 30 known cases of

tuberculosis were found to be contacts of these children, and 20 additional cases, either inactive or designated as pending, were found. It is evident that there is a relatively high yield of new tuberculosis cases found among both adults and children who were contacts of tuberculin-positive children in the kindergarten and first grade. It is especially worth while to do studies of the contacts of tuberculin "converters" since among this group, although rather small, the yield was 5.6 per cent new cases of active tuberculosis.

To sum up, it appears that the tuberculin testing of young children in the kindergarten and first grade and subsequent follow-up studies of these children and their household contacts constitute a relatively good method for the uncovering of new cases of tuberculosis.

DISCUSSION

The data presented above offer interesting evidence on which to base programs for the finding of tuberculosis in the community. The method of skin testing of kindergarten and first-grade children, with follow-up of their contacts, yields a high percentage of new cases of active tuberculosis when compared with other methods. Although the yield is not so high as that found by follow-up of contacts of active cases of tuberculosis (3 to 6.6 per cent) (3, 4), it is more than ten times as high as the yield obtained by mass roentgenographic surveys (1.8 per cent compared with 0.1 per cent) (5).

It is especially worthy of note that, among the adult contacts of tuberculin "converters," more than 5 per cent of new cases of active tuberculosis were found. Although the number of persons in this group is small, it does appear that the findings deserve the attention of others who are concerned with the development of programs for the control of tuberculosis. The method is easy to use and could easily be incorporated in many currently operating programs.

SUMMARY

Analysis is presented of the results obtained by tuberculin skin testing among kindergarten and first-grade children, with roentgenographic and family follow-up studies. These analyses indicate that:

The percentage of active new cases of tuberculosis discovered by follow-up of household contacts of tuberculin-positive children is quite high, approximately 1.8 per cent.

Although the number of persons studied to date is small, the percentage yield of new cases of active tuberculosis (5.6 per cent) which results from the follow-up of household contacts of "converters" to the tuberculin skin tests appears to be higher than that obtained by any other method.

The observed tuberculin conversion rate among 2,421 children was 0.8 per cent per year. The theoretical rate calculated for a previous cross-sectional study was 0.6 per cent per year.

The rate of reaction to tuberculin by sections of the city directly parallels the death rate for tuberculosis.

SUMARIO

Los Coeficientes de Virajes de Reacciones a la Tuberculina-Histoplasmina en la ciudad de Kansas City como Indicación de la Frecuencia de la Infección; Informe Preliminar: I. Estudios con la Tuberculina

Analízanse los resultados obtenidos con la cutirreacción a la tuberculina entre los niños de las escuelas de párvulos y de primer grado, junto con estudios radiográficos y familiares subsiguientes. Indican dichos análisis que:

El porcentaje de nuevos casos activos de tuberculosis descubiertos por medio de la observación de los contactos caseros de niños tuberculinopositivos es bastante alto, aproximadamente de 1.8 por ciento.

Aunque es pequeño el número de personas estudiado hasta la fecha, el porcentaje (5.6) de nuevos casos de tuberculosis activa descubiertos con el examen de los contactos caseros de los que acusan virajes de las cutirreacciones a la tuberculina parece ser más alto que el obtenido con ningún otro método.

El coeficiente de viraje a la tuberculina observado entre 2,421 niños fué de 0.8 por ciento al año. El coeficiente teórico calculado para un estudio transversal anterior fué de 0.6 por ciento al año.

El coeficiente de reacción a la tuberculina en los distintos barrios de la ciudad paralela la mortalidad por tuberculosis en los mismos.

RESUME

Les taux de conversion tuberculine-histoplasmine à Kansas City comme indice de la prédominance de l'infection; Rapport préliminaire: I. Etudes sur la tuberculine

Présentation des analyses de résultats obtenus chez les enfants des jardins d'enfants et des classes enfantines par les tests cutanés à la tuberculine combinés aux examens radiologiques et la surveillance du milieu familial. Ces analyses indiquent que:

Le pourcentage de nouveaux cas de tuberculose en activité, qui ont été découverts dans le milieu familial par la recherche des "contacts" des enfants ayant une réaction positive à la tuberculine, est très élevé, approximativement 1,8 pour 100.

En dépit du nombre réduit des sujets étudiée à ce jour, le pourcentage des nouveaux cas de tuberculose en activité (5,6 pour 100), qui a été obtenu à la suite de la recherche de "contacts" dans le milieu familial des enfants présentant une conversion des tests cutanés, est plus élevé que celui fourni par toute autre méthode.

Le taux de conversion de la réaction à la tuberculine observé chez 2.421 enfants est de 0,8 pour 100 par an. Le taux théorique calculé d'après une statistique antérieure pour l'ensemble des quartiers était de 0,6 pour 100 par an.

Le taux de réaction à la tuberculine par quartiers de la ville est parallèle au taux de mortalité par tuberculose.

Acknowledgments

The authors wish to thank Dr. Michael L. Furcolow, Senior Surgeon, and Mr. Myron J. Willis, Senior Assistant Sanitarian (R), Communicable Disease Center, Public Health Service, for their assistance in the analysis of the data.

REFERENCES

The references for the present paper are listed at the end of the succeeding paper.

TUBERCULIN-HISTOPLASMIN CONVERSION RATES IN KANSAS CITY AS AN INDICATION OF THE PREVALENCE OF INFECTION—PRELIMINARY REPORT[1,2]

II. Histoplasmin Studies

MICHAEL L. FURCOLOW, MYRON J. WILLIS, LAWRENCE E. WOOD, AND HERBERT L. MANTZ

(Received for publication May 19, 1953)

The annual conversion rate to histoplasmin among the general population has been reported upon only once (6). No follow-up observations on the "converters" were included. The present studies were undertaken to determine the frequency of serologic and roentgenographic changes among children whose histoplasmin skin tests changed from negative to positive under observation.

MATERIAL AND METHODS

In addition to the routine follow-up as indicated for the positive tuberculin reactors, all children who converted from a negative to a positive histoplasmin skin test had serologic tests for histoplasmosis. These tests were done by Dr. Joseph Schubert of the Communicable Disease Center Serological Laboratory, Atlanta, Georgia, and Dr. Samuel Salvin of the Rocky Mountain Laboratory, Public Health Service, Hamilton, Montana. The histoplasmin was lot H-42, furnished by Dr. C. E. Palmer of the Public Health Service. It was employed in a dilution of 1:100.

RESULTS

In figure 1 is shown the histoplasmin sensitivity among kindergarten children in the public schools, based on tests of approximately 13,000 children during the years 1947–1952. It is interesting to note that the rate of positive reaction by school district varies from less than 5 per cent to 24 per cent. This distribution of histoplasmin sensitivity is quite marked by sections of the city. High rates are found predominantly in the north and especially in the northeast section. It is to be noted that a majority of the schools showing the highest rates are located along the course of the Blue River, which is indicated on the right side of the figure by the winding water course.

In table 1 is shown the prevalence of histoplasmin reactors by sections of the city for different years. It is of considerable interest to note that the north sections are consistently higher than the south sections and that the southwest section is consistently the lowest. The consistency of the rates from year to year by section of the city is evident.

[1] From the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Kansas City, Kansas, and Atlanta, Georgia; the Kansas City Tuberculosis Society, Kansas City, Missouri; and the Tuberculosis Division, Kansas City, Missouri, Health Department.

[2] Presented before the Medical Session, as part of the session on *Nontuberculous Diseases of the Chest*, at the annual meeting of the National Tuberculosis Association, Los Angeles, California, May 19, 1953.

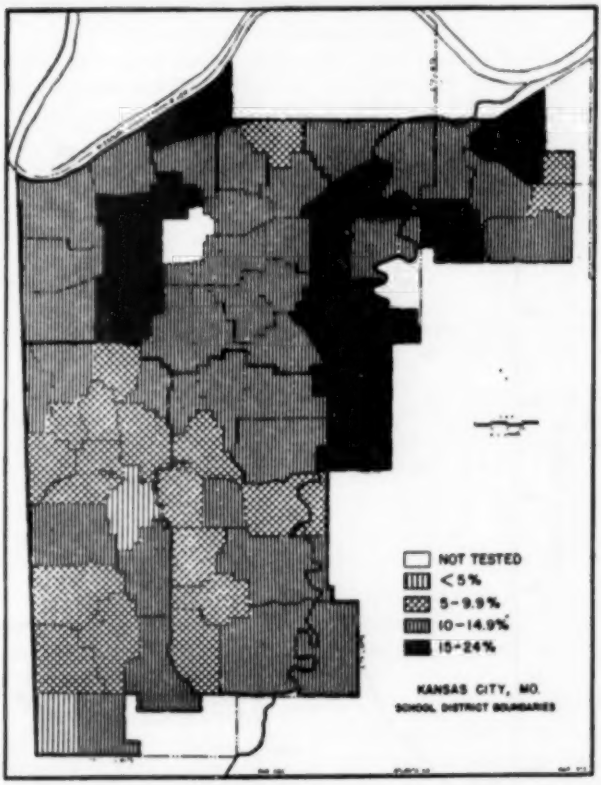

FIG. 1. Histoplasmin sensitivity among public school kindergarten children, 1947–1952.

TABLE 1

COMPARISON OF PERCENTAGE* OF POSITIVE HISTOPLASMIN SKIN TESTS AMONG 13,158 KINDERGARTEN CHILDREN BY SECTION OF THE CITY AND YEAR OF TESTING, KANSAS CITY, MISSOURI

YEAR	SECTION					RATES FOR YEARS
	Northeast	North Central	Northwest	Southeast	Southwest	
1947–1948	20.9	13.6	15.3	10.0	9.4	12.9
1948–1949	11.8	12.1	12.4	12.3	9.4	11.4
1949–1950	16.9	15.2	14.1	9.6	8.7	12.5
1950–1951	13.7	13.9	13.7	9.9	9.6	11.8
1951–1952	13.2	12.2	12.4	10.6	7.7	10.9
Section rates.....	15.1	13.5	13.5	10.4	8.9	11.9

* Each percentage shown is based on observation of 220 or more children.

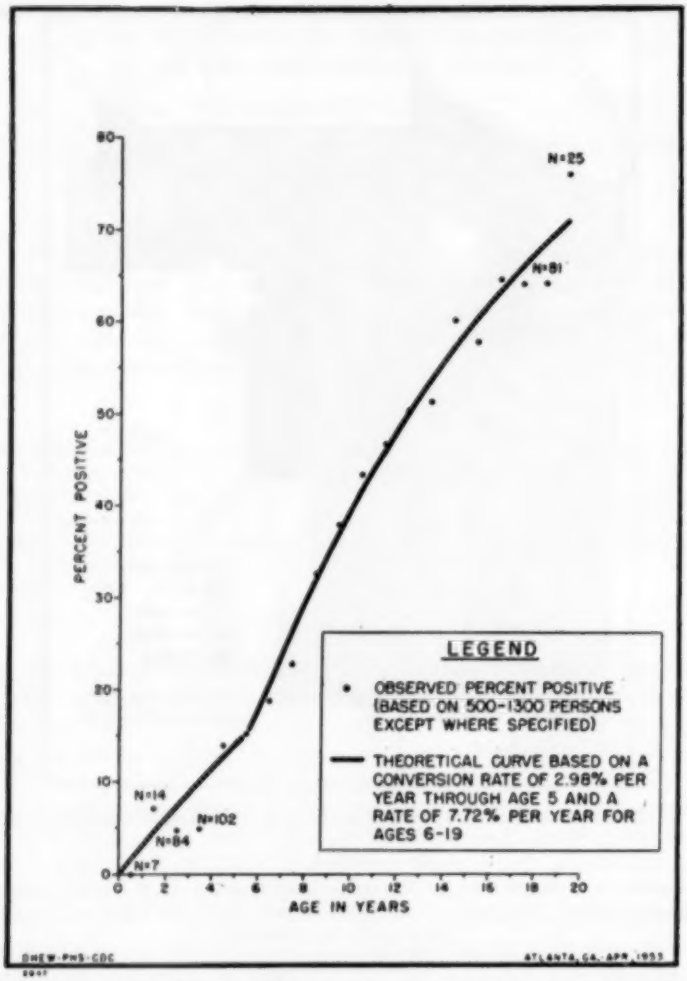
PERCENT POSITIVE
AGE IN YEARS
N=25
N=81
N=14
N=102
N=84
N=7
LEGEND
OBSERVED PERCENT POSITIVE (BASED ON 500-1300 PERSONS EXCEPT WHERE SPECIFIED)
THEORETICAL CURVE BASED ON A CONVERSION RATE OF 2.98% PER YEAR THROUGH AGE 5 AND A RATE OF 7.72% PER YEAR FOR AGES 6-19

FIG. 2. Observed and theoretical histoplasmin sensitivity rates by age among Kansas City, Missouri, children (1945 survey).

In figure 2 are shown the results obtained from the large survey performed in 1945 on approximately 17,000 Kansas City schoolchildren, which has been reported previously (1). Histoplasmin sensitivity, as indicated by the dots on the figure, is quite prevalent. The lines on the figure represent the theoretical conversion rate (Ross method (2)), which explains the observed sensitivity rates.

It was found that two rates were necessary to explain these observations. It is evident that a histoplasmin conversion rate of 2.98 per cent per year during

TABLE 2

"CONVERTERS" TO HISTOPLASMIN TEST

Number and Percentage of Children Whose Histoplasmin Tests Were Negative in Kindergarten and Positive in the First Grade One Year Later. Two Years of Observation

SCHOOL YEAR BEGINNING		SECTIONS OF THE CITY					TOTAL
		North-east	North Central	North-west	South-east	South-west	
1950	Number of negatives re-tested	196	227	110	329	268	1,130
	Number of "converters"	12	9	3	11	10	45
	Per cent	6.1	4.0	2.7	3.3	3.7	4.0
1951	Number of negatives re-tested	240	244	90	295	305	1,174
	Number of "converters"	4	2	3	4	6	19
	Per cent	1.7	0.8	3.3	1.4	2.0	1.6
Combined years	Number of negatives re-tested after one year	436	471	200	624	573	2,304
	Number of "converters"	16	11	6	15	16	64
	Per cent	3.7	2.3	3.0	2.4	2.8	2.8

the first six years of life, followed by a rate of 7.72 per cent per year from ages six to nineteen, would serve to explain the observed figures. The theoretical conversion rate, therefore, is approximately 3 per cent per year during the first six years and 7.7 per cent thereafter.

In table 2 are shown the results of retests of histoplasmin-negative children during two school years in Kansas City. As indicated, during the school years 1949–1950 and 1950–1951, 1,130 children who previously were histoplasmin negative were retested. Of these, 45 (4.0 per cent) were found to be "converters" or new reactors. During the next school year 1,174 previously negative children were retested, of whom 19 (1.6 per cent) were found to be "converters." For the two years, 64 of a total of 2,304 children (2.8 per cent) were histoplasmin "converters." This figure of 2.8 per cent is of considerable importance to note since the theoretical figure applying during this age-period of approximately five to six years was 2.98 per cent as shown in figure 2. Therefore, a combination of the observed rates for the two years fairly well approximates the theoretical histoplasmin conversion rate.

It should be emphasized that the rates for different years are quite different; that is, one year the rate is more than twice that observed in the following year. While this had been suspected for histoplasmosis, it was of interest that it could be shown by observation in children. In spite of the fact that rates are quite variable from year to year, the over-all rate is reasonably comparable to that which might have been expected.

In table 3 are shown the results obtained by follow-up of the histoplasmin-positive children. As stated previously, no attempt was made to follow the adult

TABLE 3

FOLLOW-UP STUDIES OF HISTOPLASMIN-POSITIVE CHILDREN IN KANSAS CITY, MISSOURI (1952–1953)

HISTOPLASMIN-POSITIVE CHILDREN		SEROLOGY				ROENTGENOGRAM					
		Positive	Doubtful	Negative	Total	Active	Healing	Questionable	Calcified	Negative	Total
"Converters"	Number	4	4	7	15	1	1	4	1	9	16
	Per cent	27	27	46	100	6	6	25	6	57	100
Positive on first test (kindergarten and first grade)	Number	12	18	21	51	10	7	6	13	18	54
	Per cent	24	35	41	100	19	13	11	24	33	100

contacts of these children. The table is divided into two sections, the upper section showing the follow-up observations on 16 "converters" to the histoplasmin skin test. Serologic tests for histoplasmosis were completed on 15 of these children. Four (27 per cent) showed a positive reaction. An additional 4 showed a doubtful reaction, and 7 were negative on the serologic study.

Roentgenographic examination of this group revealed lesions which were designated as active in 6 per cent, healing in 6 per cent, and calcified lesions or normal findings in 63 per cent.

Among the 54 kindergarten and first-grade children whose first histoplasmin skin tests were positive, as shown in the lower part of table 3, 12 (24 per cent) showed positive complement fixation tests; an additional 18 (35 per cent) showed a doubtful reaction. Only 41 per cent showed a negative test. Ten (19 per cent) of the group of children showed active lesions of histoplasmosis on roentgenographic examination; an additional 7 (13 per cent) showed healing lesions. Only 18 (33 per cent) of the group showed no abnormalities on roentgenographic examination.

It is of interest to note that only half of the children who had a positive histoplasmin skin test showed some serologic evidence of infection. It is also interesting to note that more than one-fourth of the children showed evidence of unhealed lung lesions, and approximately one-quarter showed evidence of calcified lesions. This is a very high prevalence of both active lesions of histoplasmosis and calcification in such a young age group.

DISCUSSION

In addition to the studies in tuberculosis reported in the first paper in this series, the present approach yields considerable dividends in terms of active lung lesions caused by *Histoplasma capsulatum*. Since the area where histoplasmosis is prevalent encompasses a sizable part of the country, the findings of the present survey would appear to have widespread application.

The finding of a high percentage of positive histoplasmosis serologic tests

and of lung lesions in young children indicates the high prevalence of active disease. While the treatment of fungal infections admittedly is poor at the present time, there is every reason to anticipate that, with the rapid development of antimicrobials, new agents will be found which will be active against fungi.

The differentiation of fungal diseases from tuberculosis is extremely important in view of the mental trauma inflicted upon patient and family by a diagnosis of tuberculosis. It is perhaps even more important that the patient not be incapacitated and put at bed rest because of a false diagnosis of tuberculosis when a fungal infection, actually present, might be adequately followed and observed without limitation of activities. Since the finding of lung lesions in the area where histoplasmosis is prevalent is common among young children, it is especially important that the physicians in the area be educated to the differential diagnosis of these lesions. Tools are available in the skin tests for the easy differentiation of these two diseases; in addition, the serologic tests offer an easy way of determining the activity of the fungal infection. Follow-up by roentgenographic and serologic tests is quite simple and practicable and offers a ready prognostic and diagnostic aid. In the education of the physicians and the public to these diagnostic tools, the tuberculosis society can play a prominent part.

SUMMARY

Analysis is presented of results obtained by histoplasmin skin testing among kindergarten and first-grade children, with roentgenographic and serologic follow-up. These analyses indicate that:

The rate of reaction to histoplasmin by sections of the city is different from that observed with tuberculin but, in general, is consistent during the five years of observation.

The observed histoplasmin conversion rate among 2,304 children was 2.8 per cent per year. The theoretical rate calculated from a previous cross-sectional study was 2.98 per cent per year for the first six years of life and 7.72 per cent per year thereafter.

Both lung lesions and positive serologic tests were observed frequently among the histoplasmin-positive children.

These preliminary results indicate the usefulness of such a program in areas where both tuberculosis and histoplasmosis are prevalent.

SUMARIO

Los Coeficientes del Viraje de las Reacciones a la Tuberculina-Histoplasmina en Kansas City como Indicación de la Frecuencia de la Infección; Comunicación Preliminar: II. Estudios de la Histoplasmina

Este análisis comprende los resultados obtenidos con las cutirreacciones a la histoplasmina en niños de las escuelas de párvulos y de primer grado, mantenidos después en observación roentgenográfica y serológica. Indican esos análisis que:

El coeficiente de reacciones a la histoplasmina en los distintos barrios de la ciudad es distinto del observado con la tuberculina, pero, en general, es constante para los cinco años de observación.

El coeficiente observado de viraje de la reacción a la histoplasmina fué de 2.8 por ciento al año. El coeficiente teórico calculado a base de un estudio de un grupo transversal de población fué de 2.98 por ciento para los primeros seis años de vida y de 7.72 por ciento al año después.

Entre los niños histoplasmino-positivos, observáronse frecuentemente tanto lesiones pulmonares como serología positiva.

Esos resultados preliminares indican la utilidad de obras de esa naturaleza en zonas en las que abunda tanto la tuberculosis como la histoplasmosis.

RESUME

Les taux de conversion tuberculine-histoplasmine de Kansas City comme indice de la fréquence de l'infection; Rapport préliminaire: II. Etudes de l'histoplasmine

Présentation d'une analyse des résultats obtenus par des tests cutanés pratiqués chez des élèves des jardins d'enfants et de la classe suivante avec examens radiologiques et sérologiques périodiques ultérieurs. Cette analyse indique que:

Le taux de réaction à la l'histoplasmine déterminé par quartiers de la ville est différent de celui enregistré pour la tuberculine, mais, en général, il est constant durant les cinq années d'observation.

Le taux de conversion de l'histoplasmine enregistré chez 2.304 enfants était 2,8 pour 100 par an. Le taux théorique, calculé d'après une enquête antérieure couvrant l'ensemble des quartiers, atteignait 2,98 pour 100 pendant les premières six années de la vie, et 7,72 pour 100 ensuite.

Les lésions du poumons et la sérologie positive étaient toutes deux fréquemment observées chez les enfants ayant une réaction positive à l'histoplasmine.

Ces résultats préliminaires indiquent l'utilité d'un tel programme dans les régions où la tuberculose et l'histoplasmose sont toutes deux très répandues.

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THE SENSITIZATION OF GUINEA PIGS TO MICROCOCCUS PYOGENES VAR. AUREUS IN THE PRESENCE OF THE "WAX" OF ACID-FAST BACILLI[1,2,3]

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INTRODUCTION

The establishment of delayed hypersensitivity to tuberculin by means other than infection was reported by Raffel (1, 2). This was accomplished by injecting guinea pigs with two isolated fractions of the tubercle bacillus, protein and the chloroform-extractable lipid or "wax." Administration of the protein alone resulted in the establishment of immediate hypersensitivity as evidenced by the anaphylactic state, but without the development of a typical tuberculin hypersensitivity. The animals which received both the lipid and protein responded to the intracutaneous injection of tuberculin by giving a typical delayed skin reaction. Myrvik and Weiser (3) have reported similar results in that guinea pigs given the crude "wax" which contained residual tuberculoprotein developed tuberculin sensitivity.

That this is not a phenomenon confined solely to the homologous system was demonstrated by Raffel and associates (4), who were able to show that the response in guinea pigs to the injection of egg albumin was altered when the "wax" of the tubercle bacillus was simultaneously injected. Animals which received the "wax" and egg albumin developed a delayed hypersensitivity to egg albumin as measured by several criteria. Raffel and Forney (5), using a similar procedure, injected guinea pigs intraperitoneally with picryl chloride alone and picryl chloride with "wax." The animals which received only the picryl chloride developed an anaphylactic state, whereas the animals which also received the "wax" of the tubercle bacillus gave a strong skin reaction to contact with the picryl chloride.

On the basis of this evidence, it appears that the "wax" of the tubercle bacillus possesses a biologic property of altering the response of an animal to injected material. To broaden the evidence still further, the present study was undertaken to study the effect of the "wax" on the injection of whole bacillary cells into guinea pigs. For this purpose, *Micrococcus pyogenes* var. *aureus* was selected. This organism is of low virulence for guinea pigs so that intercurrent natural infections would not be expected to interfere with the several experiments. In all previous studies the "wax" from human tubercle bacilli was used. In the present study the

[1] From the Department of Medical Microbiology, School of Medicine, University of Southern California, and Barlow Sanatorium, Los Angeles, California.

[2] Presented before the Medical Session, as part of the session on *Bacteriologic Research*, at the annual meeting of the National Tuberculosis Association, Los Angeles, California, on May 19, 1953.

[3] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

TABLE 1

METHOD OF SENSITIZATION

GROUP	NUMBER OF ANIMALS	SUBSTANCES INJECTED INTO GUINEA PIGS OF EACH GROUP		NUMBER OF INJECTIONS
		"Wax" used	Micrococcus pyogenes	
I	20	None	Yes	3
II	13	*M. tuberculosis*	No	3
III	21	*M. tuberculosis*	Yes	3
IV	17	*M. smegmatis*	Yes	3

"wax" from *Mycobacterium smegmatis* was used as well as "wax" from *Mycobacterium tuberculosis* var. *hominis* to determine whether or not the biologic activity of the "wax" fraction was a property of only a virulent acid-fast bacterium.

MATERIALS AND METHODS

The "wax" fractions used in this study were from two sources: *Mycobacterium tuberculosis* var. *hominis*, strain H37Rv[4]; and *Mycobacterium smegmatis*.

The organisms were grown on Youmans' modification of the Proskauer and Beck medium (6). The bacteria were killed by placing the flasks in the Arnold sterilizer for one hour. The bacillary mass was collected on coarse filter paper and let drain of excess moisture after washing with saline. The organisms were extracted in the Soxhlet apparatus for eight hours, using ethyl alcohol and ether, 1:1. The bacillary residue was then exhaustively extracted with chloroform. The chloroform extract was filtered through a Selas 03 filter to remove the bacillary sediment and finally evaporated in a stream of carbon dioxide. This product is usually termed "crude wax" (2, 3).

The strain of *Micrococcus pyogenes* var. *aureus* used was a coagulase-positive organism which had been carried as a stock culture in this laboratory and designated American Type Collection No. 152. It was cultured in tryptose phosphate broth for twenty-four hours. The bacillary mass was collected by centrifugation, killed by exposure to 1.0 per cent formaldehyde for forty-eight hours at 37°C., washed with saline, and lyophilized.

The guinea pigs were of both sexes from the colony of the Barlow Sanatorium. They were distributed so that males and females were used in each experimental group.

The materials for injection were prepared in a water-in-oil emulsion according to the method of Freund (7). The "wax" was dissolved in the paraffin oil, and the staphylococcal cells were made up in the aqueous phase. The animals which received the lipid were given 5 mg. each per injection. A dose of the staphylococcal cells was 1 mg. per animal. Each animal received three injections of the appropriate material at weekly intervals, subcutaneously in the groin.

The animals were then skin-tested eighteen days following the last injection. The skin tests were done intracutaneously on the flank, a skin-test dose consisting of 0.1 mg. of the staphylococcal cells in 0.1 ml. of saline. The animals were divided into four groups, as shown in table 1. In this way the results were controlled for possible sensitization by the "wax" alone or by the cells of the staphylococci alone.

It has been shown by several investigators (3, 4) that the corneal test may be used as a criterion for the delayed hypersensitivity state. Hence, this test was also employed in this investigation. The method used was that described by Raffel and associates (4).

Finally, each surviving animal was infected intracardially with 0.1 ml. of a suspension of *M. pyogenes* made up to match tube No. 3 of a nephelometer.

[4] A sample of the purified "wax" was kindly supplied by Dr. R. J. Anderson.

TABLE 2

ALLERGIC RESPONSE IN TREATED GUINEA PIGS

Results of Skin Tests on Guinea Pigs Receiving Injections of Various Substances

GROUP	SENSITIZING TREATMENT	NUMBER OF ANIMALS	RESULTS OF SKIN TESTS		STANDARD DEVIATION OF DIAMETER OF LESION
			Average diameter	Average increase in thickness	
			mm.	mm.	
I	Staphylococcal cells	20	3.2	0.31	2.84
II	Tubercle bacilli "wax"	13	1.3	0.006	2.05
III	Tubercle bacilli "wax" plus staphylococcal cells	21	12.2	1.8	4.6
IV	*M. smegmatis* "wax" plus staphylococcal cells	17	12.47	1.4	4.9
V	Normal controls	6	1.5	0.08	2.14

The animals were sacrificed six days later, and each animal was autopsied. The visceral organs were examined grossly, and cultures on blood agar were made of the lungs, liver, spleen, and kidneys. All organisms which grew were checked by the gram stain. In addition, samples of each of the above organs were fixed in formalin, sectioned, and stained. The stains employed were hematoxylin and eosin and MacCallum and Goodpasture's stain for bacteria in tissue.

RESULTS

The results of the skin tests are shown in table 2. The animals which received only the staphylococcal cells gave no skin reaction of any significance, being similar in this respect to the animals which received only the "wax" of the human tubercle bacillus. These results were in marked contrast to those in the animals which received the "wax" along with the staphylococcal cells.

The source of the "wax" was immaterial in that the "wax" of *M. smegmatis* was equally efficacious in establishing a delayed hypersensitivity as was the "wax" derived from *M. tuberculosis*. The standard deviation of the diameter of the skin-test lesions was calculated and indicates a significant difference in the reaction of the animals which received both the "wax" and antigen.

Corneal tests were done only on selected animals since most of the animals were to be used for other studies. The results of the corneal tests are presented in table 3. The results of the corneal tests correlate very closely with the skin tests. In a normal animal or an animal which did not receive a combination of "wax" and antigen, the only response seen in the corneal test was a pinpoint clouding as a result of trauma in performing the test. In contrast, the animals which exhibited skin sensitivity gave a definite reaction in the cornea when tested with the specific antigen, in this case whole staphylococcal cells.

The diagrammatic representation of the corneal reaction in table 3 approximates very closely the gross appearance of the cornea in each tested animal. Forty-eight hours after the test the animals were sacrificed and the eyes were removed and placed in Perenyi's fixative. Sections were made and stained with

TABLE 3

ALLERGIC RESPONSE IN TREATED GUINEA PIGS

Correlation of Skin Tests and Corneal Tests

GUINEA PIG NUMBER	SENSITIZING TREATMENT	SKIN TEST REACTION*	CORNEAL REACTION†
111	Staphylococcal cells	—	
112	Staphylococcal cells	—	
113	Staphylococcal cells	5.25	
114	Staphylococcal cells	—	
128	Tubercle bacilli "wax"	5+	
129	Tubercle bacilli "wax"	—	
130	Tubercle bacilli "wax"	—	
136	Tubercle bacilli "wax" plus staphylococcal cells	20 1.5	
140	Tubercle bacilli "wax" plus staphylococcal cells	10 1	
153	*M. smegmatis* "wax" plus staphylococcal cells	13 2.5	
158	*M. smegmatis* "wax" plus staphylococcal cells	19 3	
160	*M. smegmatis* "wax" plus staphylococcal cells	13 1.5	
A	Normal control	Negative	
B	Normal control	Negative	

* The first number indicates the diameter of the lesion in millimeters. The second number is the estimated increase in thickness of the skin expressed in millimeters.

† A diagrammatic representation of the relative size of the gross lesion of the cornea forty-eight hours after injection of staphylococcal cells.

hematoxylin and eosin. Examination of the sections revealed changes similar to those reported by Raffel and associates (4).

The results of the infections with living organisms were inconclusive. Gram-positive cocci were cultured more frequently from animals which exhibited a skin sensitivity. Grossly, these animals appeared to have more disease manifested by small suppurative foci on many of the visceral organs. Histologic examination exhibited no striking difference among the several groups.

DISCUSSION

The development of a delayed hypersensitivity to the injection of whole cells of *Micrococcus pyogenes* var. *aureus* in the presence of the "wax" extracted from acid-fast bacilli fits into the pattern set in previous reports (1–5). Weissfeiler (8) and Higginbotham (9) found that tuberculous animals which were skin-tested with *Staphylococcus aureus* gave an exaggerated inflammatory reaction. These were animals which had had no previous sensitization with the homologous antigen. In the present experiments, previous treatment with the "wax" of the tubercle bacillus did not result in any increased skin sensitivity of the animals to staphylococcal cells.

The concentration of "wax" in the animals probably exceeds that which would

be expected as a result of natural infection with the tubercle bacillus. Be that as it may, the fact remains that the response of guinea pigs to several types of antigen has been shown to be altered in the presence of the "wax" so that the "wax" would appear to possess some definite biologic activity. The specific component involved has not been clearly established although a mycolic acid-polysaccharide complex has been suggested as the active component (10).

The sensitizing ability of the "wax" of *M. smegmatis* suggests that the biologic activity may be present in all acid-fast organisms. The presence of this activity in the extract of a saprophytic organism indicates that it is in no way associated with virulence.

The role of the hypersensitivity of *M. pyogenes* herein demonstrated in subsequent infection with the homologous organism is one of interest. The degree of infection in animals previously sensitized to staphylococcal cells appears to be greater than in animals exhibiting little or no sensitivity. However, due to the native resistance of guinea pigs to infection with this organism, the results were inconclusive and will require further study. The failure of clinicians to observe in tuberculous patients any increased susceptibility to infection with nonacid-fast bacteria indicates that such a decrease in resistance in the face of a hypersensitivity does not exist in the natural state in man.

SUMMARY

From the present experiments it may be concluded that guinea pigs can be rendered hypersensitive to whole cells of *Micrococcus pyogenes* var. *aureus* when the "wax" of *Mycobacterium tuberculosis* var. *hominis* or *Mycobacterium smegmatis* is administered simultaneously. This hypersensitivity can be demonstrated by an increased skin reactivity to injection with staphylococcal cells and by a reaction in the cornea following injection directly into that tissue.

SUMARIO

La Sensibilización de los Cobayos al Micrococcus Pyogenes var. Aureus en Presencia de la "Cera" de Bacilos Acidorresistentes

A juzgar por los experimentos descritos, cabe deducir que puede hipersensibilizarse a los cobayos a células íntegras del *Micrococcus pyogenes* var. *aureus* cuando se les administra simultáneamente la cera del *Mycobacterium tuberculosis* var. *hominis* y del *Mycobacterium smegmatis*. Puede descubrirse esa hipersensibilidad por medio de la hiperreactividad cutánea a la inyección de células estafilocócicas y de la reacción observada en la córnea consecutivamente a la inyección directa en dicho tejido.

RESUME

La sensibilisation du cobaye au Micrococcus pyogenes var. aureus en présence de "cire" de bacilles acido-résistants

A la suite de cette expérimentation on peut conclure à la possibilité de sensibiliser le cobaye aux cellules entières du *Micrococcus pyogenes* var. *aureus* quand la cire du *M. tuberculosis* var. *hominis* et du *M. smegmatis* est administrée simultanément. Cette hypersensibilité peut être démontrée par l'accroissement de la réactivité cutanée à l'injection de cellules staphylococciques et par une réaction cornéenne à la suite de l'injection directe dans ce tissu.

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CLINICAL AND HISTOPATHOLOGIC STUDY OF THE EFFECTS OF ANTIMICROBIAL THERAPY IN TUBERCULOSIS[1,2]

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INTRODUCTION

Serial biopsies of tuberculous lesions in the human patient provide valuable material for study of the effectiveness of various types of therapy. Although the lung is not readily studied by this means, mucous membrane lesions (mouth, pharynx, and larynx) are readily accessible for such observations. In a previous study (1) of this type, the writers have demonstrated that treatment of mucous membrane lesions with cortisone or corticotropin was followed by worsening of the lesion and increase in the number of tubercle bacilli. In the present study, a comparison was made of the effects of the various antimicrobial drugs now used in the treatment of tuberculosis. It was believed that the information obtained would be helpful in the understanding of tuberculous disease in pulmonary or other tissues.

METHOD

Twenty-four patients with pulmonary tuberculosis (almost all far advanced and cavitary) were studied. All presented tuberculous lesions of the hard palate, pharynx, or larynx. The patients were maintained on the usual Sanitarium regimen. Six of the patients reported are those who previously received cortisone or corticotropin.

Color photographs of the mucous membrane lesions were obtained at the beginning of treatment in each case and at various intervals thereafter. Biopsies (1 to 4 pieces of tissue each) of the lesions were also obtained at the beginning of treatment and at intervals during and after therapy. Other studies performed included erythrocyte sedimentation rates, serial roentgenograms of the chest, sputum examinations, and studies of the drug resistance of the strains of *M. tuberculosis* which were isolated.

Fifty-eight biopsy specimens were studied by means of numerous sections stained by hematoxylin and eosin stain, Masson trichrome stain for fibrous tissue, and Ziehl-Neelsen stain for acid-fast bacilli.

The 6 patients previously reported received dihydrostreptomycin and para-aminosalicylic acid (PAS) following their cortisone or corticotropin treatment. The patients were treated as follows:

Dihydrostreptomycin and PAS	13 patients	31 biopsies
Streptomycin and PAS	2 patients	6 biopsies
Dihydrostreptomycin	1 patient	2 biopsies
PAS	1 patient	2 biopsies

[1] From the Municipal Tuberculosis Sanitarium, Chicago, Illinois.

[2] Presented before the Medical Session, as part of the session on *Medical Aspects of Tuberculosis*, at the annual meeting of the National Tuberculosis Association, Los Angeles, California, May 18, 1953.

Amithiozone	1 patient	3 biopsies
Isoniazid	5 patients	11 biopsies
Dihydrostreptomycin, PAS, and isoniazid	1 patient	3 biopsies
	24 patients	58 biopsies

The patients were observed at frequent intervals during therapy. When gross healing had occurred or maximum benefits seemed to have been effected, another photograph was made and a biopsy was obtained. This was taken adjacent to the pretreatment specimen. The usual period of treatment with streptomycin, dihydrostreptomycin, PAS, or isoniazid was at least four months and usually very much longer. The period of treatment with amithiozone was two months because of failure of improvement. In some of the patients it was possible to take subsequent sections at several intervals, the longest period of observation being twenty-three months.

RESULTS

The first change to occur in the patients with tuberculous ulceration of the pharynx and larynx under antimicrobial therapy was subjective (2). There was cessation of pain, frequently within forty-eight to ninety-six hours after the start of therapy. This relief of pain often occurred before there was any objective evidence of improvement. Thereafter there was a gradual decrease in edema and infiltration followed by epithelization of ulcerated areas. This improvement is usually most rapid following the use of streptomycin or dihydrostreptomycin (3). Isoniazid was next in the order of effectiveness. PAS was much slower in action and required a longer period to achieve the same degree of improvement. Amithiozone was ineffective in the single case studied. It will be recalled (1) that the patients who received cortisone or corticotropin showed no objective evidence of improvement and some became worse while under treatment.

Histopathology

Biopsy material obtained before treatment: Examination of pretreatment biopsy specimens revealed some variation in the histologic picture, but virtually all showed distinct tubercle formation with caseation necrosis, epithelioid cells, Langhans'-type giant cells, and lymphocytes.

Ulceration of the epithelium was a common finding. Edema of the stroma was variable in amount. The vascular pattern of the stroma was somewhat altered depending upon the amount of inflammatory reaction. In those areas in which the inflammation was quite pronounced, the blood vessels appeared to be fewer in number than in the relatively normal areas. Study of the fibrous connective tissue elements by Masson trichrome stain disclosed only that this particular tissue component was absent in the areas of inflammation. Where the tubercles were discrete there appeared to be some condensation of the connective tissue, perhaps due to compression by the expanding tubercles rather than proliferation of fibrous tissue.

Acid-fast bacilli were demonstrated in 7 of the pretreatment biopsy specimens and occurred most frequently in those which exhibited widespread inflammatory changes. Three of the 7 patients had had no previous treatment; 4 were patients

of the previously reported group who had recently received cortisone or corticotropin. A multitude of typical tubercles were studied without finding any organisms.

Biopsy material obtained during or following treatment: Biopsies were performed at various intervals during therapy with the antimicrobial drugs. In those patients who showed a good clinical response (alleviation of pain, diminution in swelling of the involved tissues, et cetera), biopsy specimens obtained approximately three months after beginning therapy revealed the following picture:

> The epithelial layer was regenerated but varied somewhat in thickness and in some instances displayed considerable acanthosis. Active tubercles were still evident in the stroma of patients in whom the lesions grossly appeared to be healed. These tubercles were usually quite discrete, showed little or no caseation necrosis, and were made up predominantly of epithelioid cells with a few giant cells and lymphocytes. Acid-fast bacilli were demonstrated in only one post-treatment biopsy specimen. Masson trichrome stain revealed some increase in the connective tissue which varied considerably with the degree of healing. The vascularity of the tissues was, likewise, quite variable.

Biopsy specimens obtained at longer intervals after the beginning of therapy (up to twenty-three months) showed greater proliferation of fibrous tissue and diffusely scattered, and/or collections of, lymphocytes. The epithelial layer exhibited some irregularity in its pattern of growth.

Comparison of the effects of the different antimicrobial agents used revealed only the fact that healing of the disease was more rapid and complete in patients treated with streptomycin, dihydrostreptomycin, or isoniazid than with PAS or amithiozone. No specific difference could be noted in the type of response obtained with streptomycin, dihydrostreptomycin, or isoniazid. In one patient treated solely with PAS for seven months, healing occurred but was very slow by comparison with the former drugs, and tubercles persisted. Amithiozone alone was used for less than two months in one patient. Examination of biopsy specimens showed evidence of progression while under therapy. This patient was therefore treated with dihydrostreptomycin and PAS and obtained a good result.

Representative Cases

The essential findings in representative cases in each group may be summarized as follows:

Case 1. Laryngeal tuberculosis: streptomycin and PAS (figures 1 and 2). Seven weeks after therapy, examination of biopsy material revealed an intact epithelium of uniform thickness, some edema of the stroma, and collections of epithelioid cells, but a paucity of giant cells and peripheral lymphocytes. The tubercles seemed to "blend in" with the surrounding stroma. Some lymphocytes were noted apart from the tubercles. There was no apparent increase in the fibrous connective tissue. After an interval of twenty-three months, a marked proliferation of fibrous tissue was noted and collections of lymphocytes were still present in the stroma; the irregularity of the epithelium persisted.

Case 2. Laryngeal tuberculosis: dihydrostreptomycin and PAS (figure 3). Examination of the biopsy specimen obtained three and one-half months after the beginning of therapy

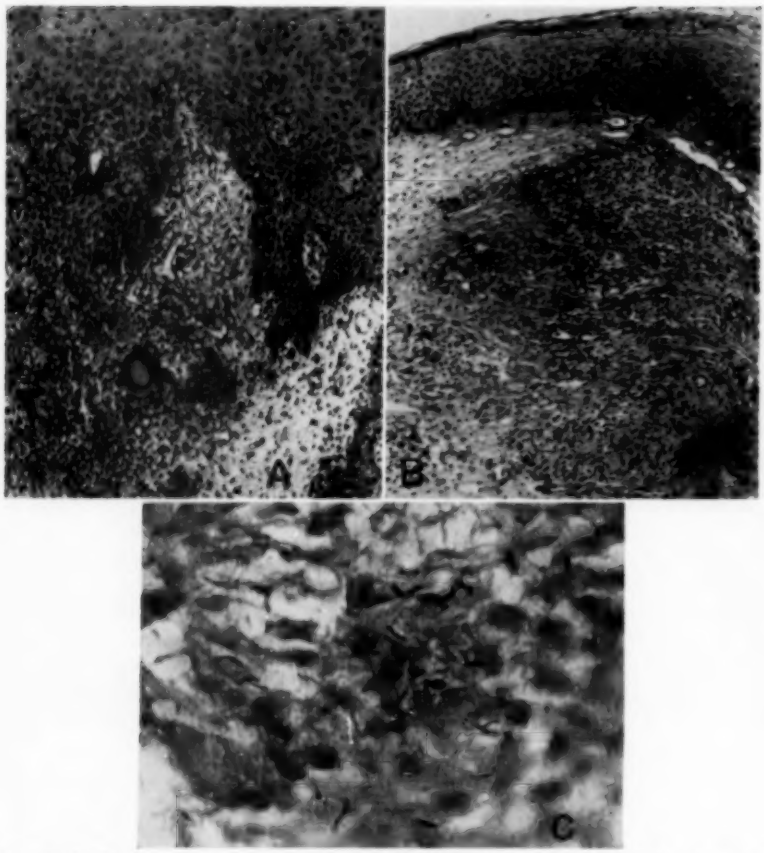

FIG. 1. J. W., white male, 39 years of age. Started on streptomycin and PAS, given daily for two weeks and then twice weekly.

A. Pre-treatment biopsy specimen (January 31, 1953) showing some acanthosis and a solitary tubercle with a large giant cell in the central portion. (H and E stain, × 100)

B. Biopsy specimen (March 21, 1953) after seven weeks of streptomycin and PAS. The epithelial layer is now regular in appearance; however, there are several epithelioid tubercles present but no giant cells or caseation necrosis. Note the scattered lymphocytes and proliferation of fibrous connective tissue. (H and E stain, × 100)

C. Acid-fast stain on the tissue from the pretreatment biopsy showing many acid-fast bacilli. (× 1000)

showed an epithelial layer of varied thickness with some acanthosis. Scattered lymphocytes were present throughout the stroma, and Masson stain showed patchy proliferation of fibrous tissue.

Case 3. Laryngeal tuberculosis: dihydrostreptomycin and PAS (figure 4). The first post-treatment biopsy specimen obtained approximately four months after the beginning of

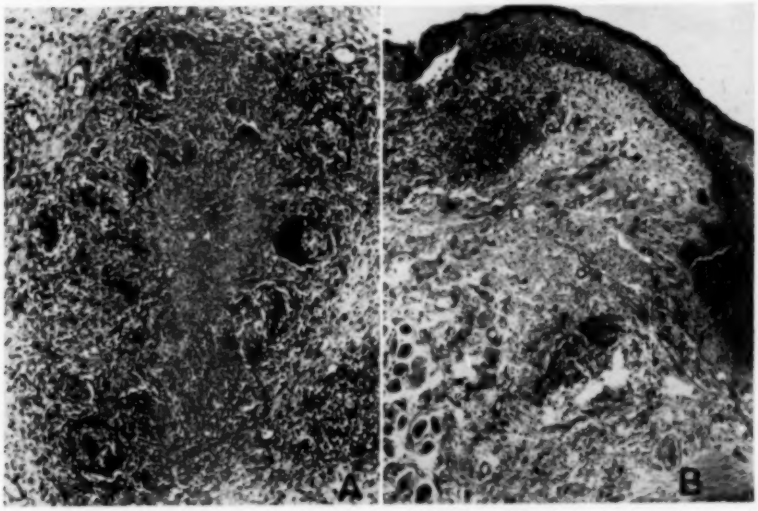

FIG. 2. J. C., white female, 25 years of age, admitted on March 24, 1951, and started on streptomycin and PAS therapy which was continued until the present time.

A. Pretreatment biopsy specimen (March 26, 1951) showing coalesced tubercles in the stroma. There is considerable caseation necrosis and there are numerous Langhans'-type giant cells present. (H and E stain, × 100)

B. Biopsy specimen obtained after twenty-three months of streptomycin and PAS therapy, showing irregularity of the epithelial layer, marked proliferation of fibrous connective tissue, and collections of lymphocytes. Some muscle cells are seen in the lower left-hand corner. (H and E stain, × 100)

therapy with dihydrostreptomycin and PAS showed an intact epithelium and considerable fibrous tissue proliferation of the stroma, some of which had undergone hyalinization. Beneath the epithelium and extending down into the stroma, collections of chronic inflammatory cells were noted. The second biopsy specimen was obtained approximately four months later and revealed some acanthosis of the epithelium along with variations in thickness. Several lymphocytes were seen scattered through the stroma with little tendency toward aggregation. Again some proliferation of fibrous connective tissue was seen. Approximately three weeks later, the therapy was changed to isoniazid and PAS, and the final biopsy specimen obtained four months later showed some acanthosis of the epithelium and many discrete to confluent tubercles in the stroma. Little or no caseation was noted, but many typical giant cells were present. Connective tissue stain revealed complete destruction in the vicinity of the tubercles. Section stained for acid-fast bacilli showed some organisms occurring singly and quite apart from each other.

Case 4. Laryngeal tuberculosis: isoniazid (figure 5). After five months of therapy the biopsy revealed a covering epithelium of various thicknesses, showing considerable acanthosis. In the stroma many scattered lymphocytes were seen, and special staining revealed considerable increase in connective tissue.

Case 5. Laryngeal tuberculosis: PAS (figure 6). A biopsy was performed six months after treatment solely with PAS, and microscopic examination of the specimen revealed that

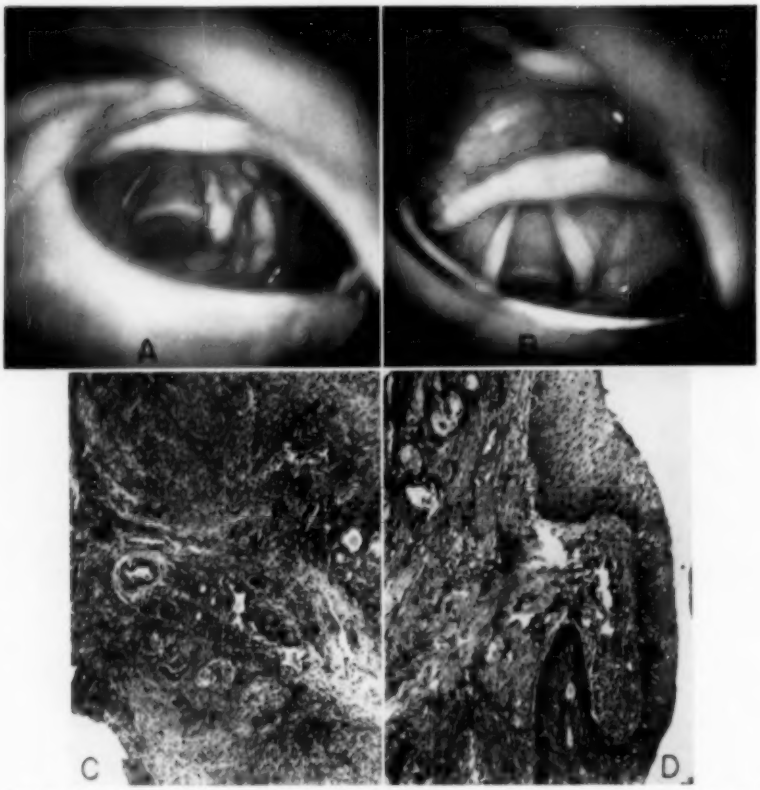

FIG. 3. J. Gu., white male, 69 years of age, admitted on February 26, 1951. Given dihydrostreptomycin from March 8, 1951 to June 20, 1951, and dihydrostreptomycin and PAS from June 22, 1951, to November 24, 1951.

A. Mirror appearance of the larynx on March 3, 1951. There is extensive ulceration and infiltration of the left hemilarynx.

B. Appearance on June 23, 1951, after dihydrostreptomycin therapy. The ulceration has healed; only slight hyperemia and mucosal thickening persists.

C. Pretreatment biopsy specimen (March 6, 1951) showing ulceration of the epithelium and a marked cellularity of the stroma. A large tubercle is noted in the lower left-hand corner and there are scattered inflammatory cells throughout the section. Note the preservation of some glands and blood vessels. (H and E stain, × 100)

D. Post-treatment biopsy specimen (June 25, 1951) showing a regeneration of the epithelium and a disappearance of the epithelioid tubercles. There are a few scattered lymphocytes as well as some proliferation of fibrous tissue.

FIG. 4. P. S., white male, 43 years of age, admitted to the hospital on January 18, 1952. Dihydrostreptomycin and PAS, started on January 29, 1952, daily for two weeks then twice weekly to October 14, 1952; isoniazid and PAS from October 14, 1952, to date.

A. Mirror appearance on January 19, 1952, revealed marked thickening of the epiglottis with superficial ulceration. The rest of the larynx is hidden but is diffusely involved.

B. Appearance on September 13, 1952, after eight months of therapy. The epiglottis is quite normal as is also the rest of the larynx.

C. Note the ulceration of the epithelium and replacement by granulation tissue in the pretreatment biopsy specimen (January 19, 1952). There is a diffuse inflammatory reaction in the underlying stroma. (H and E stain, × 100).

D. After four months of dihydrostreptomycin and PAS, a biopsy specimen (May 24, 1952) shows re-epithelialization and an occasional collection of cells simulating a tubercle. There

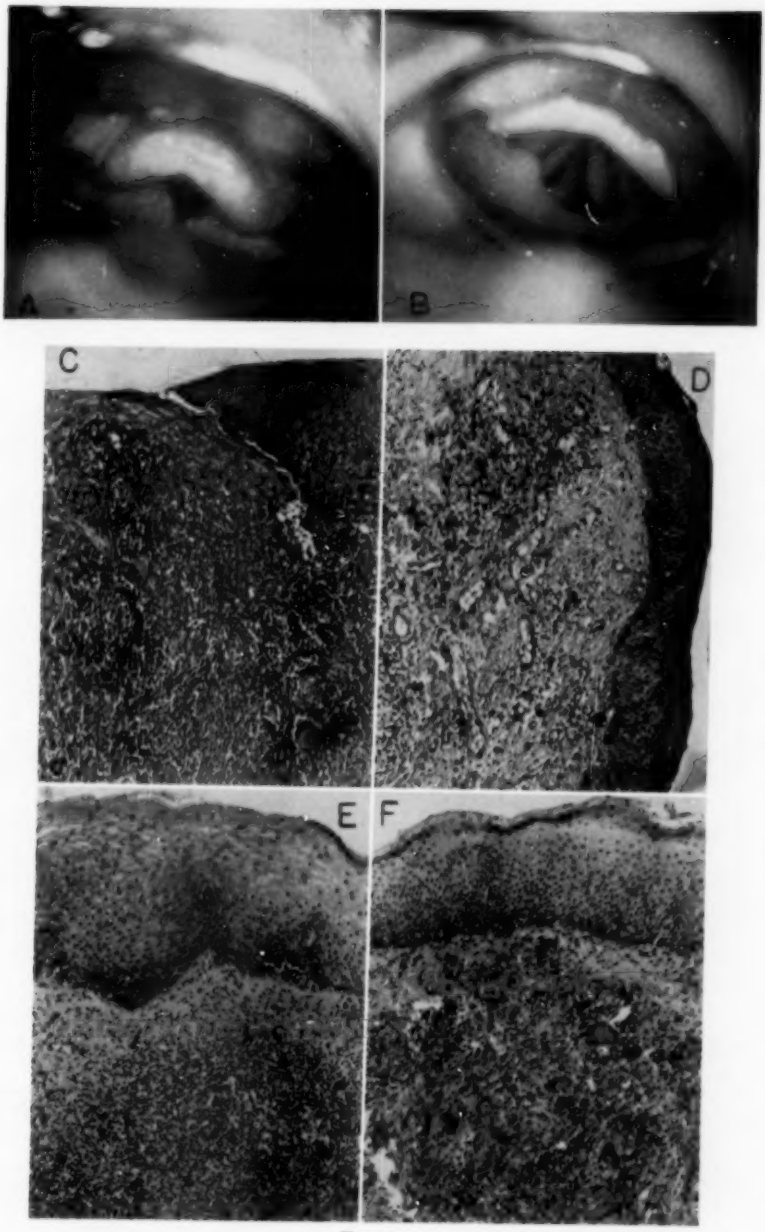

FIG. 4

is a marked decrease in the cellularity of the stroma and there is some proliferation of fibrous tissue. (H and E stain, × 100)

E. Biopsy specimen (September 27, 1952) showing some increase in the thickness of the epithelial layer and a marked increase in the number of lymphocytes. No tubercle formation could be demonstrated. (H and E stain, × 100)

F. This biopsy specimen was obtained after four months of isoniazid and PAS (February 21, 1953) and shows an intact epithelium but the reappearance of many confluent tubercles in the stroma. Note the typical giant cells present. (H and E stain, × 100)

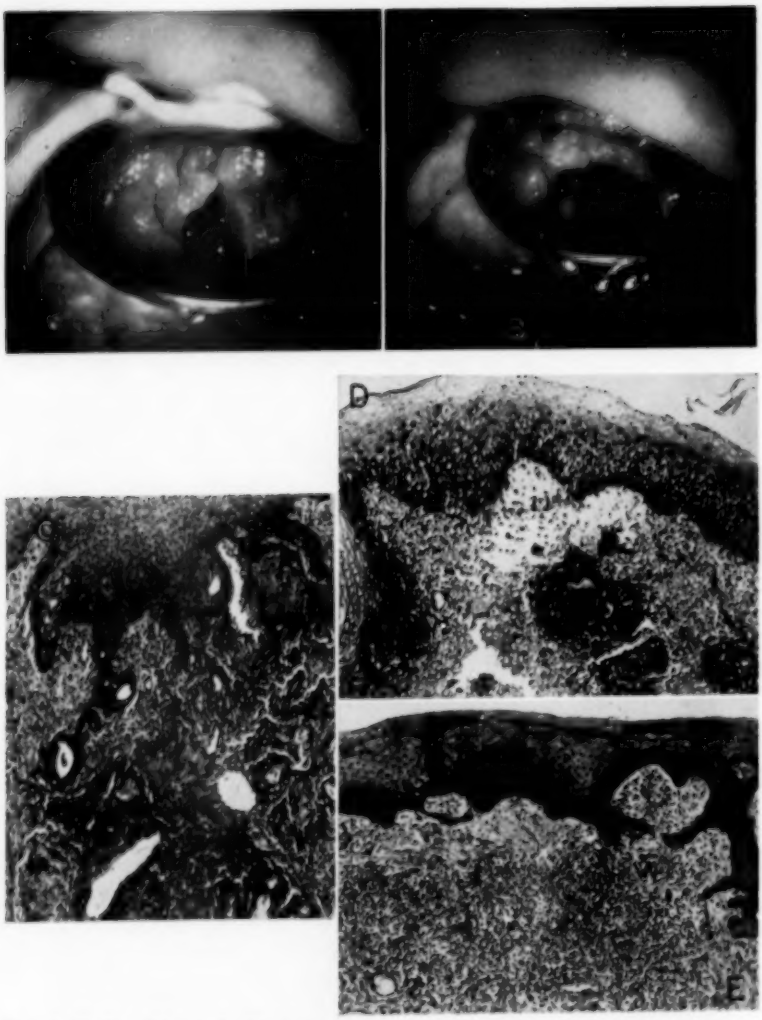

FIG. 5. E. B., white male, 31 years of age, admitted on December 28, 1951. Started on isoniazid therapy on March 9, 1952, and continued until October 25, 1952.

A. Mirror appearance on March 8, 1952, reveals marked alteration of the epiglottis. There is a V-shaped segment missing and the edges are greatly thickened and ulcerated with exuberant granulations.

B. Appearance on August 16, 1952, after isoniazid therapy. The missing segment is larger and the edges are not as thick. Healing of the ulceration has taken place and the granulations have largely disappeared.

C. Pretreatment biopsy specimen (March 8, 1952) showing some acanthosis of the epithelium, several epithelioid tubercles, and scattered inflammatory cells. (H and E stain, × 100)

D. Another area of the same biopsy specimen. Note the edema of the stroma and the huge giant cells present. (H and E stain, × 100)

E. Biopsy specimen (August 16, 1952) after continuous therapy with isoniazid. The tubercles have disappeared and there is pronounced increase in the fibrous connective tissue. The epithelium displays some acanthosis and many lymphocytes are noted. (H and E stain, × 100)

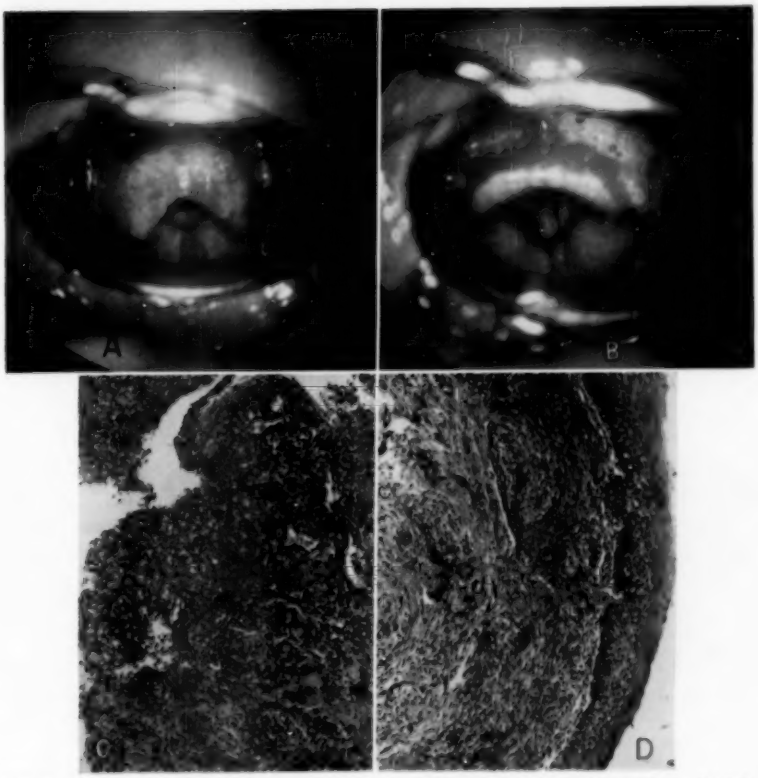

FIG. 6. L. B., white male, 24 years of age, admitted on March 28, 1951. Given PAS from April 15, 1951, to November 2, 1951, when he was discharged against medical advice.

A. Mirror appearance on April 14, 1951, revealing a marked alteration of the epiglottis. It is greatly thickened and ulcerated, hiding the rest of the larynx from view; the latter was diffusely infiltrated and ulcerated.

B. Appearance on October 13, 1951 after PAS therapy. The epiglottis is about normal in appearance; the only remaining alteration is diffuse thickening of the arytenoids and aryepiglottic folds.

C. Pretreatment biopsy specimen (April 16, 1951) showing marked ulceration of the epithelium and replacement by granulation tissue with many confluent tubercles in the stroma. (H and E stain, × 100)

D. Biopsy specimen (October 15, 1951) following treatment with PAS. Note the regenerated epithelial layer; however, epithelioid tubercles are still present in the stroma. There is no caseation necrosis and only one giant cell is seen. There is evidence of fibrous tissue proliferation.

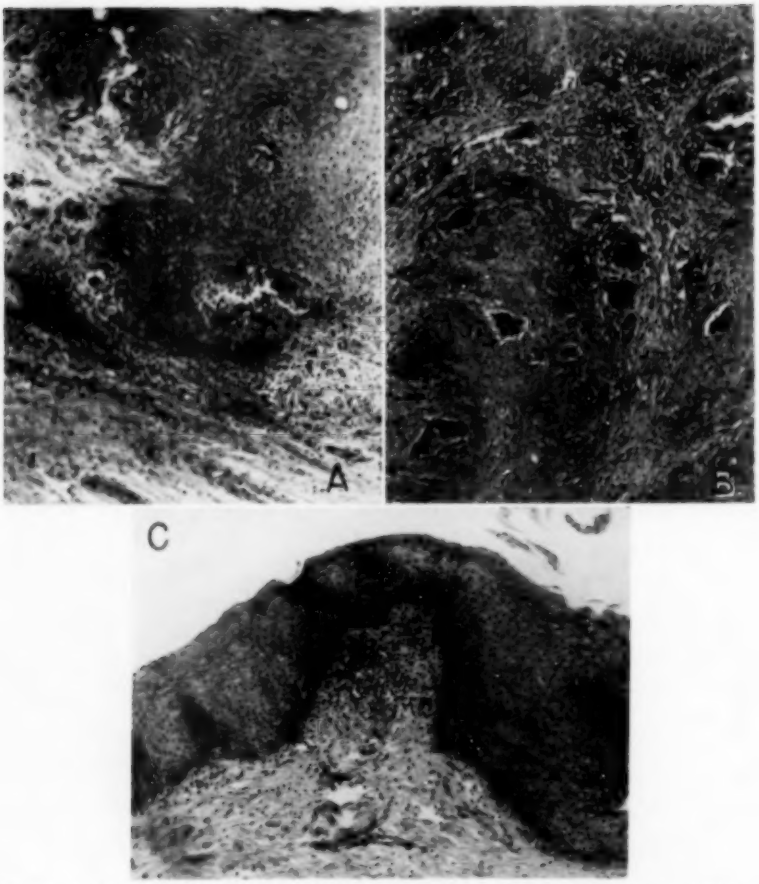

FIG. 7. J. G., white male, 47 years of age, admitted on June 6, 1951. Given amithiozone from July 16, 1951, to August 27, 1951, then dihydrostreptomycin and PAS from August 28, 1951, to February 28, 1952.

A. Pretreatment biopsy specimen (July 7, 1951) showing marked irregularity of the epithelial layer with a solitary tubercle in the central portion. Scattered lymphocytes are seen throughout the stroma. (H and E stain, × 100)

B. Biopsy specimen (August 27, 1951) following amithiozone therapy. Note the marked increase in the number of tubercles which have become confluent and show many giant cells and some caseation necrosis. (H and E stain, × 100)

C. Final biopsy specimen (August 9, 1952) approximately five months after the completion of dihydrostreptomycin and PAS therapy. The epithelial layer varies in thickness and several lymphocytes are noted immediately below it, but the outstanding feature is the marked proliferation of fibrous tissue. (H and E stain, × 100)

the epithelium was intact but varied somewhat in thickness. Several discrete tubercles were noted in the stroma. The tubercles were composed largely of epithelioid cells and showed only a small amount of necrosis, a paucity of giant cells, and a few lymphocytes. With special stains, it was possible to demonstrate an increase in connective tissue apart from, surrounding, and at points invading, the tubercles. Sections stained for acid-fast bacilli failed to show any organisms present.

Case 6. Laryngeal tuberculosis: amithiozone (figure 7). After seven weeks of amithiozone therapy, marked progression of the disease process was noted, as evidenced by the histopathologic demonstration of extensive involvement with confluent tubercles showing many giant cells and some caseation necrosis. The overlying stratified squamous epithelium and the stromal vessels were not unusual. Masson trichrome stain revealed an absence of connective tissue in the involved stroma. Multiple therapy (dihydrostreptomycin and PAS) was then given and a biopsy specimen, obtained almost one year later, disclosed some irregularity of the epithelial layer, scattered lymphocytes, and a minimal increase in fibrous connective tissue.

Summary of histopathologic findings: From the sections studied, it was evident that treatment with streptomycin, dihydrostreptomycin, or isoniazid resulted in a disappearance of active tubercles after approximately four months of therapy (4). Subsequent biopsies showed only more proliferation of fibrous tissue, the presence of few to many lymphocytes, and variations in the epithelial layer (5). One patient who was treated with isoniazid after prolonged treatment with dihydrostreptomycin did not show the favorable response in the lesions which was seen in those patients treated initially with isoniazid. While some fibrous tissue proliferation was noted along with a diminution in the number of tubercles seen in these patients treated with PAS alone, there was still considerable evidence of activity and a marked reduction in the number of giant cells. Treatment of a single case with amithiozone for two months resulted in progression of the disease.

Ultimate Outcome of the Patients Studied

The study was begun early in 1951. During the subsequent two years, 5 of the 24 patients had died of progressive pulmonary tuberculosis. Two were discharged with arrested disease, and one with inactive disease. Eight left the hospital against medical advice. The remaining 8 patients still have active disease and are still under treatment.

Examination of serial roentgenograms usually showed some improvement of the pulmonary lesions during the first few months of antimicrobial therapy. Later the pulmonary lesions progressed to a fatal termination in 5 patients, although the laryngeal lesions healed and remained healed. Post-mortem examination in 3 cases revealed healed laryngeal lesions, although the patients had been discharging tubercle bacilli in the sputum during the terminal period.

DISCUSSION

It is evident that treatment with any of the effective antimicrobials produces healing of the gross tuberculous lesion, followed after a variable period of time by healing of the microscopic lesion. The sequence of events seems to be as follows: disappearance of tubercle bacilli, diminution in inflammation, dis-

appearance of tubercles, and, finally, replacement of necrotic material by fibrous connective tissue.

Morphologically, the results attained with streptomycin, isoniazid, PAS, or combinations of these, were the same except for the time required to attain maximum improvement. Streptomycin and dihydrostreptomycin were found to give the quickest improvement. Isoniazid was also very effective, but somewhat slower. It is noteworthy that isoniazid, although very effective as an initial therapy, was relatively ineffective in a patient who had previously received considerable dihydrostreptomycin (figure 4). This coincides with the experience of the present writers that isoniazid does not usually cause clearing of the roentgenographic abnormalities in patients who have residual disease after maximum improvement on streptomycin. Para-aminosalicylic acid was considerably slower in producing healing, and microscopic evidence of persisting disease remained for a number of months after therapy was started. Amithiozone was of doubtful value since progression of disease could be noted in the single patient who received this drug.

Apparent healing of the macroscopic lesion may occur while tubercles persist in the underlying stroma. If tubercles persist, it appears highly probable that living tubercle bacilli are still present whether they are demonstrable or not. As long as living tubercle bacilli are present, relapse of the lesion remains a possibility, and it follows, therefore, that the lesion should not be considered healed until tubercles have disappeared.

After morphologic healing of the tubercles, the laryngeal lesion has usually remained healed even in those patients whose pulmonary lesions were progressive with a fatal end result. The diminution in numbers or disappearance of tubercle bacilli from the sputum while the patient is under chemotherapy is probably an important contributory factor in preventing reinfection of the larynx. Nevertheless, it is still difficult to explain the relative speed and thoroughness of healing in the larynx as contrasted with that in the pulmonary lesion. The difference in healing between the two tissues may lie in the relative solidity of the laryngeal tissue; in the greater number of tubercle bacilli present in the pulmonary lesion; in the greater amounts of caseous material present in the pulmonary lesion; or in the fact that cavitation is common in the lung.

The laryngeal lesion attracts attention early because of pain or hoarseness. If untreated, it often causes serious constitutional deterioration by interfering with deglutition. The symptoms and findings, therefore, are usually exaggerated in comparison with the small size of the lesion. These facts lead to early recognition and treatment of laryngeal lesions. This contrasts with the frequent delay in the treatment of pulmonary lesions, which leads to massive involvement with caseation necrosis and cavitation. It may be that results in the treatment of pulmonary lesions would be as good as with laryngeal lesions if treatment were given at an early stage while involvement was very limited in extent; however, differences in local defense mechanisms cannot be ruled out as causes of the different results of therapy.

The effectiveness of the antimicrobial drugs is in marked contrast to the ill

effects observed after ten to twenty-two days of treatment with cortisone or corticotropin. The ill effect of these substances is considered to be the result of their effects upon the host. This interference with the defense mechanism is reflected within a few weeks in an increase in the number of tubercle bacilli noted in the mucosal lesion. Under treatment with antimicrobial drugs, the tubercle bacilli disappear and the host soon heals the lesion. Since it has been demonstrated that isoniazid penetrates readily into caseous areas in the lungs (6), it would be assumed that bacteriostasis should be just as effective in the lung as in the larynx. However, the failure of the lung to heal as readily as the larynx indicates either a local inactivation of the drug or a local failure of the defense mechanism.

The use of biopsies of mucous membrane lesions is recommended for the study of the progress of tuberculous disease as it is influenced by a variety of factors in the human patient. The biopsy can be used as an indicator of the effectiveness of medication on the tubercle, which is basically the same whether in the lung or elsewhere.

SUMMARY

Serial photographs and biopsies were made of the accessible mucous membrane lesions of 24 patients under antimicrobial therapy. The common sequence of events with effective therapy was as follows: subjective relief, healing of the gross lesion, disappearance of tubercle bacilli, diminution in microscopic inflammation, resolution of tubercles, and fibrous tissue replacement. Dihydrostreptomycin, streptomycin, and isoniazid were rapidly effective. Para-aminosalicylic acid was much slower; amithiozone was ineffective in a single case. The contrasting results obtained with cortisone and corticotrophin as compared with the antimicrobials is discussed. The method used is recommended as a means of studying the tubercle in the human patient.

SUMARIO

Estudio Clínico e Histopatológico de los Efectos de la Terapéutica Antimicrobiana en la Tuberculosis

Obtuviéronse fotografías y biopsias seriadas de las lesiones accesibles en las mucosas de 24 enfermos que recibían terapéutica antimicrobiana. La evolución más común de los acontecimientos con una terapéutica eficaz fué la siguiente: alivio subjetivo, cicatrización de la lesión macroscópica, desaparición de los bacilos tuberculosos, disminución de la inflamación microscópica, resolución de los tubérculos y substitución de tejido fibroso. La dihidroestreptomicina, la estreptomicina y la isoniacida se mostraron rápidamente eficaces. El ácido para-aminosalicílico fué mucho más lento, la amiotozona resultó ineficaz. Discútense los resultados diversos obtenidos con la cortisona y la córticotropina, comparadas con los antimicrobianos. Recomiéndase el método usado como medio de estudiar el tubérculo en los seres humanos.

RESUME

Etude clinique et histo-pathologique des effets de la thérapie antibactérienne dans la tuberculose

Des photographies et biopsies en série de lésions accessibles des muqueuses ont été faites chez 24 malades soumis au traitement antibactérien. La suite habituelle des évène-

ment, lorque la thérapie était efficace, se manifestait par: une amélioration fonctionnelle, la cicatrisation des lésions, la disparition des bacilles tuberculeux, la réduction des lésions inflammatoires microscopiques, la résolution des tubercules et l'apparition de tissu fibreux cicatriciel. L'action favorable de la dihydrostreptomycine, de la streptomycine et de l'isoniazide se manifestait rapidement. L'acide para-aminosalicylique agissait plus lentement. L'amithiozone était inefficace. Les résultats opposés obtenus avec la cortisone et l'hormone corticotrope, par comparaison avec la thérapie antibactérienne, sont étudiés. Les auteurs recommandent la méthode utilisée comme un moyen d'étude du tubercule dans la tuberculose humaine.

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EFFECTS OF ISONIAZID AND IPRONIAZID ON THE CENTRAL NERVOUS SYSTEM OF THE DOG[1]

WILLIAM SCHALLEK AND DONALD WALZ

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INTRODUCTION

Previous work indicates that large doses of isoniazid and iproniazid have toxic effects on the central nervous system of the dog. Benson, Stefko, and Roe (1) found that isoniazid, 100 mg. per kg. intravenously or orally, caused "central nervous system signs consisting of apprehension, anxiety, and convulsions, which became continuous and terminated in death." Similar symptoms were noted with iproniazid at 140 mg. per kg. administered by the same routes. Rubin and coworkers (2) found that isoniazid, 50 mg. per kg. intravenously, caused fatal convulsions in dogs. In chronic experiments, isoniazid at 10 mg. per kg. per day caused ataxia, tremors, and convulsions after ten weeks of oral administration; with higher doses, convulsions occurred more rapidly (2, 3).

In view of clinical reports of central nervous system disturbances in patients treated with these drugs (4–8), it seemed worth while to make a further study of the effects of these compounds on the central nervous system of the dog. Particular attention was paid to the minimal doses which caused neurotoxic symptoms. Observations were made of the effects of these drugs on the electroencephalogram as well as on the behavior of the dogs.

METHODS

For the acute experiments, observations of drug effects on behavior were made following intravenous injection in unanesthetized dogs. Electroencephalographic changes were observed in dogs prepared under thiopental anesthesia, using a modification of the procedure described by Schallek and Smith (9). Artificial respiration was instituted through a tracheal cannula, while a hypodermic needle was inserted in a femoral vein for injecting solutions. The electroencephalogram was recorded from steel needles inserted in holes drilled through the skull. On completion of the operative procedure, the areas around the incisions were infiltrated with procaine and the animals immobilized with decamethonium (C_{10}). Drugs were injected two hours after the last injection of thiopental. Records were taken at periodic intervals for four or five hours; a Grass Model III C electroencephalograph was used. At the conclusion of the experiment, the animal was sacrificed. For analysis of the results, the dominant frequency and amplitude were measured on typical sections of the records.

For the chronic experiments, simultaneous observations were made on behavior and electroencephalographic changes, using trained, unanesthetized dogs. Electrodes were attached to the shaved scalp with bentonite paste and then secured with collodion, using the method described in the *Instruction Manual for the Grass Electroencephalograph*. The electrodes, made by the Grass Instrument Company for clinical use, are cup-shaped discs of pure silver, approximately 8 mm. in diameter. The dogs were observed for two to three hours following oral administration of the drugs.

[1] From the Department of Pharmacology, Hoffmann-LaRoche Inc., Nutley, New Jersey.

RESULTS

Acute Experiments

Single doses of isoniazid (10, 20, and 50 mg. per kg., intravenously) had no effect on the behavior of 5 unanesthetized dogs. When these "priming doses" were followed one day later, however, by test doses of 40 or 50 mg. per kg., definite effects were produced. There was no change in behavior for the first hour following injection. The dogs then showed periods of gradually increasing hyperactivity and finally burst into tonic-clonic convulsions. The behavior returned to normal in approximately three hours. Iproniazid, which was tested on 6 unanesthetized dogs, produced less central stimulation than isoniazid. Dose levels which produced convulsions in the isoniazid dogs either had no effect or produced only hyperactivity in the iproniazid dogs (table 1).

TABLE 1

ACUTE EXPERIMENTS ON UNANESTHETIZED DOGS

(The following dogs received priming doses of 10 mg. per kg. intravenously on the day preceding the test dose. Six additional dogs, pretreated with 20 mg. per kg., showed similar results)

DOG NUMBER	DATE 1953	DRUG	TEST DOSE, MG. PER KG. INTRAVENOUSLY	EFFECT
A-6	2/5	Isoniazid	30	None
A-5	2/10	Isoniazid	40	Convulsions in 3 hours
A-4	2/3	Isoniazid	50	Convulsions in 2 hours
A-8	2/17	Iproniazid	40	None
A-9	2/18	Iproniazid	50	Hyperactive in 9 minutes
A-9	2/25	Iproniazid	60	More alert in 20 minutes; blood in urine, feces

Representative Protocols

Dog A-5, Male—10.0 kg.

2/2/53—Isoniazid, 20 mg. per kg., intravenously—no effect

2/3/53—Isoniazid, 50 mg. per kg., intravenously—no change for 1 hour

1 hour, 10 minutes—Dog became hyperactive, followed by weakness of hind legs; hypersensitive to light and sound

1 hour, 20 minutes—Emesis; dog disoriented, moved blindly about cage, throwing himself at the door; twitching of body and neck

1 hour, 30 minutes—Convulsions, tonic-clonic type

1 hour, 35 minutes—Dog lies quietly, exhausted, then becomes hyperexcitable, disoriented

2 hours, 10 minutes—Relaxed, but still shows some anxiety

3 hours—Resting, apparently normal

Dog A-9, Female—7.8 kg.

2/17/53—Iproniazid, 10 mg. per kg., intravenously—no effect

2/18/53—Iproniazid, 50 mg. per kg., intravenously—no change for 5 minutes

7 minutes—Dog excited

9 minutes—Whining, hyperactive

12 minutes—Restless, clawing at door

1 hour—Quiet, apparently normal

The effects of these drugs were next compared on the electroencephalograms of 2 dogs prepared under the thiopental-C_{10} technique described above. Doses of isoniazid which caused convulsions in unanesthetized dogs produced bursts of high-amplitude spikes in the electroencephalogram, the maximum effect occurring one to two hours after injection (figure 1). Iproniazid, on the other hand, caused only slight increases in amplitude. These data are summarized in table 2.

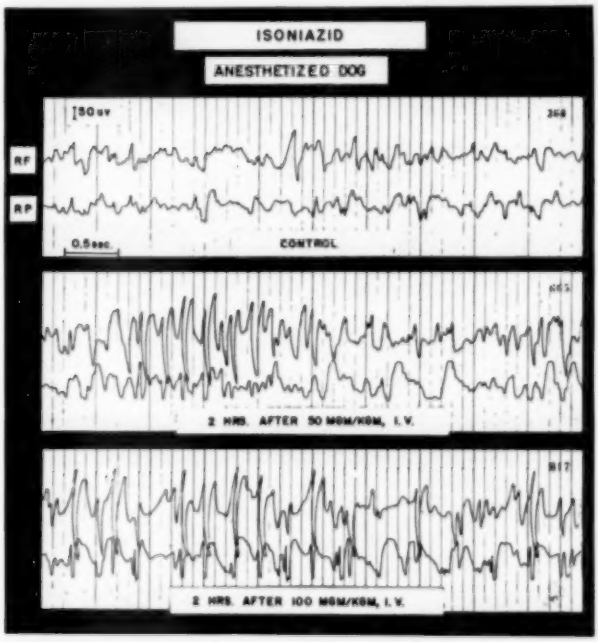

FIG. 1. Effect of isoniazid on electroencephalogram of dog prepared under thiopental-C_{10} technique. Dog. No. 267, male, 6.3 kg.

TABLE 2

EFFECTS OF DRUGS ON ELECTROENCEPHALOGRAMS OF ANESTHETIZED DOGS

DOG NUMBER	DRUG	PRIMING DOSE ON PRECEDING DAY (MG. PER KG. INTRAVENOUSLY)	TEST DOSE	MAXIMUM ELECTROENCEPHALOGRAPHIC AMPLITUDE MICROVOLTS		INCREASE	TIME IN MINUTES TO REACH MAXIMUM AMPLITUDE
				Before Drug	After Drug		
267	Isoniazid	40	50	100	350	3.5×	50
			100*	200	600	3.0	105
268	Iproniazid	60	50	130	170	1.3	180
			100*	170	200	1.2	90
245	Picrotoxin	None	0.5	100	430	4.3	11

* The 100 mg. per kg. dose was injected three hours after the 50 mg. per kg. dose.

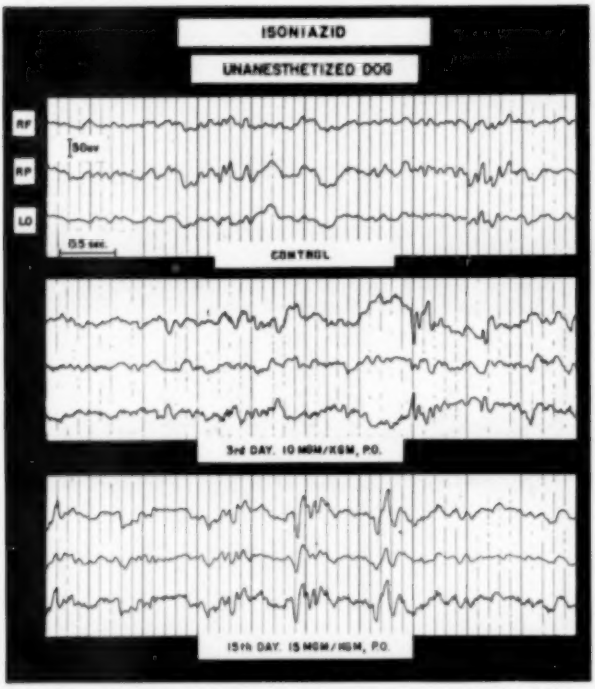

FIG. 2. Effect of isoniazid on electroencephalogram of unanesthetized dog. Trained dog No. 1, female, 6.3 kg.

Chronic Experiments

Drugs were administered to 3 dogs in daily doses of 10 mg. per kg. orally for the first ten days, 15 mg. per kg. for the next five days, and 20 mg. per kg. for the three succeeding days. None of the dogs showed any changes in behavior which could be ascribed to the action of the drugs. Dog No. 1 showed bursts of high-amplitude spikes in the electroencephalogram after three days on isoniazid (figure 2). These spikes continued for the remaining fifteen days of treatment and disappeared on withdrawal of the drug. Dog No. 2 showed spikes after eight days of isoniazid; these did not recur during the remainder of the treatment. The only symptom displayed by dog No. 3 on iproniazid was muscular twitching during the eighth day of treatment.

DISCUSSION

The central effects of isoniazid in the dog resemble those caused by picrotoxin, both in producing convulsions in unanesthetized dogs and in increasing the amplitude of the electroencephalogram in thiopental-C_{10} dogs (table 2). In regard to the doses used, however, isoniazid is much less active than picrotoxin, 50 mg. per kg. of isoniazid given intravenously producing less electroencephalographic

change than 0.5 mg. per kg. of picrotoxin. Moreover, picrotoxin exerts its effect more rapidly than isoniazid, the maximum effect at these dose levels being reached in eleven minutes with picrotoxin and in fifty minutes with isoniazid. Isoniazid in the present experiments had little central action unless a priming dose had been given on the preceding day, whereas picrotoxin did not require such priming.

The only previous report on the electroencephalographic effects of isoniazid in experimental animals seems to be that of Angelico and associates (10). These investigators found that 100 mg. per kg. of isoniazid intravenously had no effect on the electroencephalogram of the curarized rabbit. At 150 mg. per kg., a "grand mal" convulsive pattern appeared in the electroencephalogram, followed by death in twelve minutes. The investigators concluded that isoniazid has no electroencephalographic effects below lethal dosages. The present studies indicate that sublethal doses of isoniazid have definite electroencephalographic effects in the dog since spikes appeared in the electroencephalograms of 2 unanesthetized dogs which showed no changes in behavior (figure 2).

In man, "no abnormal patterns" were seen in the electroencephalograms of patients under treatment with isoniazid (4 to 10 mg. per kg. per day) or iproniazid (4 mg. per kg. per day) (11). Nevertheless, the iproniazid patients showed signs such as involuntary muscle twitching, hyperreflexia, and mild euphoria, indicating central nervous system effects of the drug. The isoniazid patients had effects qualitatively similar, but less severe and less frequent (8).

The present studies indicate that isoniazid has more central effect in the dog than iproniazid. A similar finding in schizophrenic patients is reported by Pfeiffer (12). He observed that "with an oral dose of 15 mg. per kg. of isoniazid, fourteen of thirty-one patients showed strong recruitment of the facial muscles when exposed to a flickering light for three minutes. In contrast, none of these thirty-one patients showed recruitment when tested after a 15 mg. per kg. oral dose of iproniazid."

SUMMARY

The effects of isoniazid and iproniazid have been compared on the central nervous system of the dog. Sixteen animals were used in these experiments. Isoniazid resembles picrotoxin both in causing convulsions in unanesthetized dogs and in causing bursts of high-amplitude spikes in the electroencephalograms of anesthetized dogs. Iproniazid, at the same dose levels, has much less effect on the central nervous system, the unanesthetized dogs showing transient hyperactivity and the electroencephalograms of the anesthetized dogs showing small increases in amplitude.

SUMARIO

Efectos de la Isoniacida y la Iproniacida sobre el Sistema Nervioso Central del Perro

Para comparar los efectos de la isoniacida y la iproniacida sobre el sistema nervioso central de perro, se usaron dieciséis animales. La isoniacida se parece a la picrotoxina tanto por provocar convulsiones en los perros sin anestesiar como por producir episodios de

picachos de mucha amplitud en los electroencefalogramas de los perros anestesiados. La iproniacida, a las mismas dosis, ejerce mucho menos efecto sobre el sistema nervioso central, mostrando los perros sin anestesiar hiperactividad pasajera y los electroencefalogramas de los anestesiados pequeños aumentos de la amplitud.

RESUME

Les effets de l'isoniazide et de l'iproniazide sur le système nerveux central du chien

Les effets de l'isoniazide et de l'iproniazide sur le système nerveux central du chien ont été comparés. Seize animaux ont servi à l'expérimentation. L'isoniazide ressemble à la picrotoxine, à la fois en provoquant des convulsions chez le chien non anesthésié et en déterminant l'apparition d'ondes en pointe de grande amplitude dans les électroencéphalogrammes du chien anesthésié. Aux mêmes doses, l'iproniazide a beaucoup moins d'action sur le système nerveux central; le chien non anesthésié manifeste une hyperactivité temporaire et l'amplitude des ondes est peu augmentée dans l'électroencéphalogramme du chien anesthésié.

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GENETIC STUDIES ON MICROBIAL CROSS-RESISTANCE TO TOXIC AGENTS[1, 2]

III. Cross-Resistance of *Mycobacterium Ranae* to Twenty-Eight Antimycobacterial Agents

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INTRODUCTION

It is currently established that tubercle bacilli readily develop resistance to a majority of the clinically important antimycobacterial agents. Outstanding examples of such agents are streptomycin, *p*-aminosalicylic acid (PAS), isoniazid, viomycin, and amithiozone.

Empirical evidence suggests that multiple chemotherapy may provide the simplest method to combat the emergence of resistant strains. To be effective, such therapy requires the use of at least two agents which are not related by cross-resistance. An attempt has been made, therefore, to study the development of resistance in *Mycobacterium ranae* to numerous known or potential antimycobacterial agents and to prepare a table of cross-resistance expressing the susceptibility of normal and resistant strains to all of these substances. The experiments with *M. ranae* were preceded by similar studies performed with *E. coli* (1) and *Micrococcus pyogenes* var. *aureus* (2). In addition to the determination of cross-resistance patterns as a possible contribution to antimicrobial therapy, the over-all objective of these investigations has been to establish a biologic classification of antimicrobials on the basis of their cross-resistance patterns and to determine the effect of different bacterial species upon the patterns obtained.

MATERIALS AND METHODS

A strain of *Mycobacterium ranae*[3] was used in these experiments. Bacterial stocks were prepared in Difco nutrient broth containing 0.05 per cent Triton WR-1339. Bacteria were grown at 34°C. in 15 to 30 cc. prescription bottles (Armstrong-Cork, GX, black screw-cap) and aerated by means of a wrist-action shaker until the final titer was approximately 10^9 cells per ml.

The following antimicrobial agents were employed: chlortetracycline (Aureomycin), bacitracin, catenulin, chloramphenicol, circulin, illudin M and S, licheniformin B, mycomycetin, isoniazid, iproniazid, neomycin, netropsin, amithiozone, nisin, patulin, PA-89 antibiotic, penicillin, polymyxin B, sodium *p*-aminosalicylate, streptomycins (parent, dihydro-, and hydroxy-forms), streptothricin, oxytetracycline (Terramycin), thiolutin, vinactin, and viomycin.

Seitz-filtered, sterile stock solutions were prepared, using demineralized water as solvent. All plating was done on Difco nutrient agar supplemented with 0.1 per cent yeast extract and 2 per cent glycerol. The gradient-plate technique was used both for developing resistant strains and for determining their comparative drug susceptibilities. These procedures have been described in previous publications (1, 2, 3).

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[1] From the Biological Laboratory, Cold Spring Harbor, New York.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[3] Kindly supplied by W. Steenken, Jr., Trudeau Laboratory; seventh subculture.

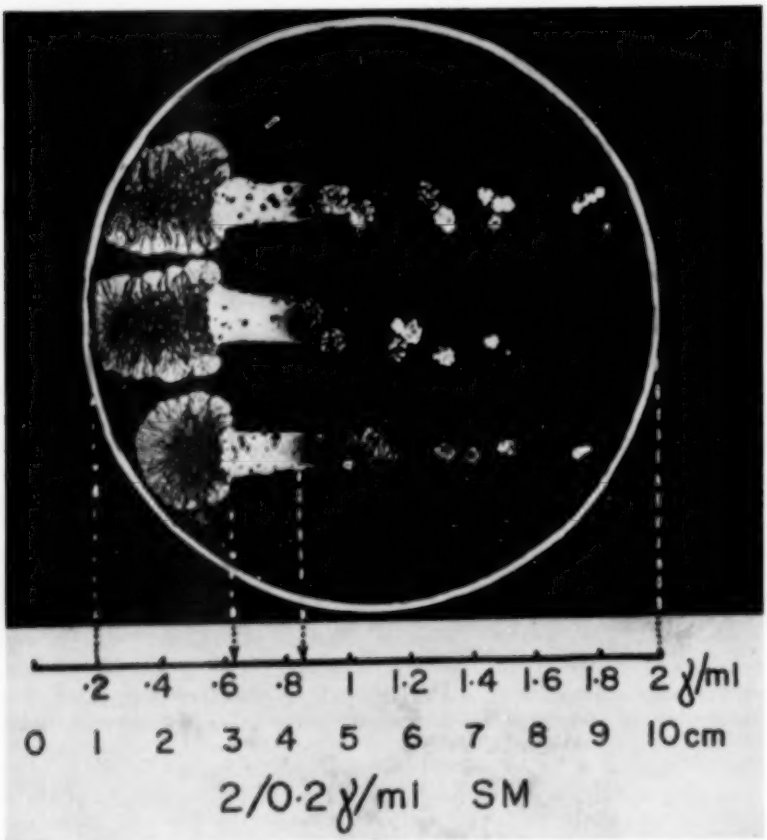

FIG. 1. Growth of *M. ranae* on a gradient plate. The plate was prepared by adding 2 γ of streptomycin per ml. in the upper agar wedge only, so that the streptomycin concentration varied along the horizontal axis of the plate from approximately 0.2 to 2 γ per ml. Seeding was accomplished by placing three streaks of a heavy bacterial suspension on the surface of the agar by means of a glass pipette. The photograph was taken after incubation of the preparation for three days at 37°C. *M. ranae* shows normal growth at streptomycin concentrations up to approximately 0.6 γ per ml. Very limited growth occurs at concentrations between 0.6 and 0.8 γ per ml. Only single, resistant colonies are able to grow beyond the 0.8 γ concentration. Between streptomycin concentrations of 0.6 to 0.8 γ per ml., the growth of resistant colonies is strongly decreased due to the competitive effect of incompletely inhibited drug-susceptible cells. As a result, small resistant colonies (dark spots on this photograph) appear on a background of whiter growth representing the numerically preponderant susceptible cells. The figure shows the principle of determining inhibitory concentrations of antimicrobial drugs by measuring the length of the growing streak. In the example given, the inhibitory concentration is 0.6 γ per ml. (photograph taken by Mr. Henry H. Jones).

In the present experiments, heavy suspensions of *M. ranae* were streaked over the surface of gradient plates because the growth of surface colonies of this organism is much more rapid than the development of submerged colonies. Figure 1 provides a typical example of mycobacterial growth on a gradient plate containing, in this instance, streptomycin. In the absence of toxic substances, colonies which arose from single cells achieved a diameter of 1 to 3 mm. after two days of incubation. In the presence of a toxic agent, resistant colonies often grew considerably slower, requiring a correspondingly longer incubation period. Plates were kept in polyethylene bags sealed with rubber bands to prevent desiccation.

RESULTS

Isolation of drug-resistant strains: The isolated drug-resistant strains could be subdivided into several classes on the basis of: frequency of occurrence of resistant cells within drug-susceptible cultures; degree of drug resistance; and presence of any noticeable abnormalities in morphology or growth rate.

Strains resistant to either streptomycin or isoniazid may develop practically complete resistance in a single step (4, 11) with hardly any alteration in the growth characteristics of many such strains. Resistance to a saturated solution of chloramphenicol also develops in a single step. In this situation, however, the resistant mutant exhibits mucoid growth. High resistance without substantially impaired growth characteristics developed in only a few steps to the following antimicrobials: licheniformin B, viomycin, vinactin, neomycin, catenulin, and illudin M and S. Low solubility of the illudins, however, limited the range of concentrations tested. Although it was possible to select strains having moderate or high resistance to bacitracin and mycomycetin, their growth was strongly impaired and the colonies had a mucoid appearance. Less rapid growth was also observed with strains exhibiting low or moderate resistance to circulin, polymyxin B, streptothricin, chlortetracycline, oxytetracycline, thiolutin, PA-89, penicillin, and PAS. It was not possible to obtain a more than twofold increase in resistance to iproniazid. No increase in resistance was noted after growing *M. ranae* on gradient plates in the presence of nisin and patulin. By restreaking the seemingly more resistant cells on the same gradient plate after preliminary incubation, it was possible to obtain some growth of *M. ranae* at twice the inhibitory concentration of amithiozone. This apparent resistance was unstable and the resulting strain, when isolated and assayed on another gradient plate, proved to be even slightly more susceptible than the parental culture.

Cross-resistance studies: Cross-resistance was determined by assaying the susceptibility of each resistant strain against the entire series of antimycobacterial agents. The figures in table 1 represent the "factor of resistance", i.e., the ratio of the susceptibilities of the resistant and parental strains. Increase in the susceptibility is shown as a fraction.

The top row in table 1 indicates the concentrations required to inhibit the parental strain of *M. ranae*. For example, it is seen from the table that the strain M/BC with fiftyfold increased resistance to bacitracin (the diagonal of the table) is six times more resistant to mycomycetin and two times more susceptible to viomycin. Therefore, the inhibitory concentrations of bacitracin,

TABLE 1

Cross-Resistance Relationship of Certain Antimycobacterial Agents as Tested with Mycobacterium Ranae

Figures represent the factor of resistance (fold increase) compared with that of the parental strain set as a unit. Values larger than one indicate an increase of resistance; fractions indicate a decrease of resistance (collateral sensitivity). The symbol 1·1 represents slightly (1·1 to 1·5 times) increased resistance; and the symbol 1/1, slightly increased susceptibility; other figures are also rough estimates. The values in the top row are the concentrations of the agents, heading each vertical column, required to inhibit the parental strain. Resistant derivatives of *M. ranae* are listed in the first vertical column in the same sequence as the antimicrobial agents heading the table; resistance is conventionally represented by a bar. Figures on the diagonal of the table show the resistance factor of each of these experimental strains to the agent used in its selection.

STRAINS	ANTIMYCOBACTERIAL AGENTS																								
	Nisin	Circulin	Polymyxin B	Licheniformin B	Streptothricin	Viomycin	Vinactin	Neomycin	Catenulin	Streptomycin	Netropsin	Chlortetracycline	Oxytetracycline	Thiolutin	PA-89	Chloramphenicol	Bacitracin	Mycomycetin	Penicillin	Patulin	PAS	Amithiozone	Iludin-M	Iludin-S	Isoniazid	Iproniazid
	Sensitivities of *M. ranae* (γ per ml.)																								
	3000	200	25	1·5	15	2	4	0·5	2	0·6	4	0·05	0·4	15	15	20	200	0·2	900	30	3000	1500	1	5	5	1500
	Factors of Resistance																								
M/NI	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1/1	1	1/1	1	1	1	1/1	1/1	1	1
M/CR	1	**4**	5	10°	1·5°	3	3	2°	3	2°	1	1	1/1	1/1	1	1·5°	1	1/2	1°	1/2	1	1/1	1	**4**	**50°**	1·5
M/PB	1	**4**	**15°**	10°	2	3	3	2	3	600	**4**	1	1/1	1/1	1	1·5	1	1/2	1	1/1	1	1/1	1	**4**	**50°**	1·5
M/LI	1/1	**4**	7	**80**	3	3	3	6	**4**	4	2	1/2	1/1	1	1	2	1	1/2	1/2	1/1	1/2	1/3	1	**4**	**10°**	1/1
M/ST	1/1	1·1	7	2°	**10**	1·1	1·1	1·5	30	1·5°	3	1	1/1	1/1	1·1	1	1/1	1	1	1/2	1	1/3	1/1	1/2	1	1
M/VM	1/1	3	1·1	3°	1·1	>**10**[3]	>10[3]	60	**500**	1·1	1	**2**	1·1	1/1	1	1/1	1/1	1	1/1	1	1	1/1	1	3°	1	1/1
M/VI	1/1	3	7	4°	3	250	**250**	>10[3]	120	3	1·5	1·5	1	1/1	1	1·1	1	1	1	1/1	1	1	1/1	1	1·1	1·1
M/NM	1/1	3	2	2°	3	2	2	>**10**[3]	**500**	3	2	1	1	1/1	1	1	1/1	1	1/2	1	1	1	1/1	1	1	1
M/CT	1/1	3	**4**	1	**4**	1·1	1·1	80	**500**	>10[3]	**4**	1/2	1/1	1/1	1	1	1	1/2	1/1	1/2	1/1	1/1	1	1	1°	1
M/SM	1	1	**4**	1	1·1°	1	1	1·1	**4**	>**10**[3]	1	1	1	1·1	1	1	1	1/2	1	1	1	1	1	1	1	1
M/NT	1/4	1	1	1/4	**4**	1	1	1	1	1·5°	**20**	1	1/1	1/4	1	1/1	1	1	1/3	1/2	1/1	1/3	1/2	1/4	80°	1
M/AU	1	1	1	2	1	1	1	1	1	1	1	**5**	5	1/1	1	1	1/2	1	1	1	1	1	1/1	1	1	1
M/TM	1	1	1	1°	1	1	1	1	1	1	1	80	**80**	1/1	1	1/1	1	1	1·5	1	1	1	1/1	1	1	1

M/TL	1	1/1	1	1	1	1	1	1	1	1	1	1	1/2	>8†	1	3*	1/1	1/1	1/1	1/2	1	1	2	1/1	1/1	1
M/PS	1	1·1*	1·1*	1·1*	1*	1*	1*	1	1	1*	4*	1*	1	1	>8†	3*	1·1	3*	2*	1·1	1	1	1·5	1·5*	1*	1
M/CM	1	1	1	1·1*	1	1	1	1	1	1	1	1·5*	1	1·1*	2*	>90†	4	5*	2*	1/1	1/1	1/2	1	4	50‡	2‡
M/BC	1	1	1/1	3*	1/2	1/2	1/2	1	1/1	1/1	1/1	1·5*	1	1	2*	10*	50	6*	2*	1·1	1	1/1	10*	3*	1	1
M/MM	1	2	1	2*	1	1	1	1·1	1·5	1	1/1	1	1	1*	2*	4*	20	2000	2*	2*	1/1	1/1	1	1·1*	1	1
M/PN	1	1	1	1	1	1	1	2	1	1	1/1	1	1	1	1	1	2	2*	8	1	1	1	1	1·5*	20*	1
M/PT	1	1	1	1·1	1	1	1	1	1	1	1	1·1	1	1	1	1	1	1	1/1	1	1	1	1/1	1/1	1	1
M/PAS	1	1	1	1	1/1	1	1	1	1	1	1/1	1	1	1	1	1	1/1	1	1/1	1	4	1	1	1	1	1
M/NTS	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1/1	1	1	1/1	1/1	1/1	1	1
M/IM	1/1	1	1	1	1·1	1	1	1	1	1*	1	1	1	1	1	1	1	1	1/1	1/2	1	1	>10†	>10†	1	1
M/IS	1	1	1	1	1·1	1	1	1	1	1*	1	1	1	1	1	1·1	1	1*	1/1	1/2	1	1	>10†	>10†	1	1
M/INH	1/1	1/2	1	1	1·1	1·1	1	1	1	1·1	1	1	1/2	1	1	1	1	1/1	1	1	1	1	1/1	1/1	800	2
M/IPH	1/1	1/2	1	2*	1·1	1·1	1	1	1	1·1	1	1	1/1	1	1	1	1·1	1/1	1	1	1	1	1/1	1/1	800	2

* Relatively high proportion of cells with a resistance factor higher than that indicated in the table.

† Growth in saturated solution.

‡ One of the six chloramphenicol-resistant strains tested had the factor of resistance equal to 1/3.

mycomycetin, and viomycin for the bacitracin-resistant *M. ranae* are 10,000 (50 × 200), 1.2 (6 × 0.2), and 1 (½ × 2), respectively.

Several facts become apparent upon studying the table of cross-resistances. The data have been tabulated so as to place in proximity the agents related by cross-resistance, and thereby to reveal their relationships.

The largest group consists of some bacterial and *Actinomyces* polypeptides together with neomycin, catenulin, streptomycin, and netropsin. The degree of cross-resistance, however, varies considerably within this group. A high degree of cross-resistance is observed between microbiologically identical viomycin and vinactin and between quantitatively distinguishable catenulin and neomycin. Strains resistant to the first two antimicrobials are also highly resistant to the latter, but the reverse relationship is of a very low order. Streptothricin, streptomycin, and netropsin are less closely related to this group, with several cases of unidirectional cross-resistance only. A slightly higher degree of cross-resistance is observed between strains resistant to circulin, polymyxin B, and licheniformin. The polymyxin-resistant strain shows a striking increase in resistance to streptomycin. Hydroxystreptomycin, dihydrostreptomycin, and streptomycin behave identically in all of these tests. Therefore, only streptomycin was included in the table.

The second group consists only of chlortetracycline and oxytetracycline, two drugs of a very similar chemical structure.

Chloramphenicol, bacitracin, mycomycetin, and PA-89 constitute a third group. Bacitracin and mycomycetin are also related to penicillin by a low degree of cross-resistance.

The illudins form a fourth group exhibiting a high degree of cross-resistance. Low solubility and a scarce supply of these substances made it impossible to determine the upper level of resistance.

The fifth group is composed of isoniazid and its derivative, iproniazid. The high natural resistance of *M. ranae* to iproniazid excluded the possibility of selecting strains with more than a twofold increase in resistance. These strains, however, were fully resistant to isoniazid.

The table does not reveal any other pronounced groups related by cross-resistance. It should be noted, however, that several resistant strains show an increase in resistance to isoniazid and illudin S.

Resistant strains of *M. ranae* do not exhibit "collateral sensitivity" (1, 2) to any high degree; a few examples of fourfold increase in susceptibility are noted.

In addition to changes in the threshold of susceptibility, resistant strains may have an altered proportion of cells resistant to another toxic agent or a different mutation rate. Striking examples of increased proportion of resistant mutants are marked with an asterisk on the table.

DISCUSSION

Results emerging from the present study are analogous to those obtained in previous experiments with *Escherichia coli* and *Micrococcus pyogenes* var. *aureus* (1, 2). There are, however, differences in many details, and a few antimicrobial agents not included in the earlier investigations have received attention.

In comparison with the two previously studied bacterial species, *M. ranae* exhibits a higher degree of selectivity within the first group of antimicrobials. The microbiologic identity of viomycin and vinactin is confirmed, but it is possible by quantitative test to detect a significant difference between the spectra of catenulin and neomycin (vertical columns, table 1). Streptomycin and netropsin are only very loosely related to this group, mainly by one-way cross-resistance as in the case of *E. coli* and staphylococci. *M. ranae* strains resistant to polymyxin B show an even greater increase in resistance to streptomycin than do the other two bacterial species (1, 2).

Strains of *M. ranae* resistant to chlortetracycline and oxytetracycline are completely cross-resistant; this species apparently is not able to distinguish between the two drugs. As in the case of *M. pyogenes* (2), but contrary to the experience with *E. coli*, it was found that strains of *M. ranae* resistant to chlortetracycline and oxytetracycline did not show any appreciable cross-resistance relationship to other antimicrobial agents. Netropsin is again (2) more closely related to the first group and is not related to chlortetracycline-oxytetracycline as is the case with *E. coli* (1).

The third group of agents related by cross-resistance is composed of bacitracin, mycomycetin, chloramphenicol, and PA-89. Penicillin also shows some slight affinity to this group, which includes drugs of varied origin, properties, and presumably chemical structure. It is difficult to estimate the relationship of cross-resistances to possible similarities in the mechanism of antimycobacterial action of the drugs within this group. With minor exceptions, *M. pyogenes* and *E. coli* do not show any appreciable cross-resistance between the antimicrobials of group three, i.e., strains of these bacterial species resistant to one agent are not resistant to other agents within the group. It may be concluded that grouping is, to some extent, species specific.

Complete cross-resistance was noted between illudin M and S, two antimicrobial drugs produced by the basidiomycete *Clitocybe illudens*. They were not previously studied with *E. coli* and *M. pyogenes*.

Exposure of *M. ranae* to isoniazid or iproniazid results in the selection of strains highly resistant to isoniazid. As the inhibitory activity of iproniazid is originally very low, however, little further increase in resistance to its action may be obtained. There is no cross-resistance between strains resistant to isoniazid and to other potent antimycobacterial agents such as streptomycin, viomycin, PAS, and amithiozone. The high natural resistance of saprophytic mycobacteria to the two latter agents diminishes the significance of this observation.

Several other investigators, however, report a similar lack of cross-resistance, both *in vitro* (5, 6, 7, 8) and *in vivo* (9, 10), for pathogenic tubercle bacilli resistant to isoniazid, on one hand, and to streptomycin, PAS, and amithiozone, on the other. This correlation between *in vitro* studies with *M. ranae*—and both *in vitro* and *in vivo* experiments with other mycobacteria including *M. tuberculosis*—may be further exemplified by the reported lack of substantially increased resistance of streptomycin-resistant strains to neomycin (11, 12, 13), viomycin (14, 15, 16, 17, 18, 19), PAS (20, 21), and licheniformin (22).

Resistance to streptomycin is either increased two- to threefold in strains

resistant to licheniformin, subtilin (22), and viomycin (23) or decreased one- to sixfold in neomycin- (11, 18) and amithiozone- (24) resistant strains. It could be observed that several unrelated antimicrobials select *M. ranae* with significantly increased resistance to isoniazid. When screening several strains resistant to chloramphenicol, five of the six independently isolated cultures had increased resistance to isoniazid. One strain, however, was three times more susceptible to this drug than the parental *M. ranae*. All of these chloramphenicol-resistant stocks were isolated as one-step mutants, capable of growth in medium saturated with the drug. Since full resistance of *E. coli* to chloramphenicol develops in several steps (26, 27) but requires only one step with *M. ranae*, there appears to be an apparent contradiction to the earlier hypothesis of Bryson and Demerec (25) that the pattern of resistance development depends on the nature of the agent used and is not related to the bacterial species. This disparity does not necessarily invalidate the hypothesis if it be assumed that the relatively insusceptible wild type *M. ranae* exists at a resistance level close to the potential maximum. Any other speculation on the genetic makeup of this species must remain unanswered until it is possible to conduct crossing experiments similar to those performed with *E. coli* by Cavalli and Maccacaro (27).

On the basis of the previous observations with *E. coli* and *M. pyogenes* (1, 2) and the present investigation, it is possible to conclude that a high degree of "collateral sensitivity" is a rather rare phenomenon. "Collateral sensitivity" (1) is said to occur when a strain resistant to one agent exhibits a considerably higher susceptibility to another agent than does the parental strain. A low degree of "collateral sensitivity," on the other hand, is very often encountered and may be one of the contributing factors in the complex phenomena of synergism between various antimicrobials as described by Klein and Schorr (30), Dowling, Lepper, and Jackson (31), Jawetz and Gunnison (32), and Szybalski (33).

Two other properties of resistant strains are also noticeable. One is a small degree of increased resistance to unrelated agents; the other is a change in the average proportion of cells resistant to one drug within a strain selected for resistance to another. Both properties presumably have changed as a result of the preferential selection of physiologically different types of cells and are not thought to depend on the inductive influence of the drug *per se*.

These incidental changes lend further support to the general observation that the isolation of bacteria with modified genetic composition by selective agents has several effects. One obviously is the establishment of strains which differ in response to the selective agent itself. Another is the correlated appearance of secondary changes in the biologic properties of the strain; for example, modifications in growth rate and morphologic appearance. The incidental alterations observed presumably arise because few, if any, mutations produce only one change; more commonly there is one major effect and several minor ones. Future research may continue to reveal that many resistant strains are altered in nutritional requirements or virulence and that antimicrobial drugs related by cross-resistance select for resistance mutants with similar primary and secondary

properties. In this study, the term mutant has been applied to bacteria with heritable differences in resistance, in the absence of precise localization of the responsible genetic factors within the chromosomes or other carriers of heredity. Demonstration that resistance markers behave as point mutations has been advanced in bacterial strains amenable to sexual crossing (27, 28, 29) and is strong presumptive evidence of a similar mechanism in mycobacteria. The significance of prolonged adaptive changes in drug susceptibility, if any, cannot be assessed from the type of investigation herein presented.

SUMMARY

Using the "gradient-plate" technique (3), strains of *Mycobacterium ranae* were selected, almost completely resistant in a single step to streptomycin, isoniazid, and chloramphenicol; highly resistant to licheniformin B, mycomycetin, viomycin, vinactin, neomycin, and catenulin; moderately resistant to illudin M and S, bacitracin, polymyxin B, streptothricin, netropsin, chlortetracycline (Aureomycin), oxytetracycline (Terramycin), thiolutin, PA-89, and penicillin; and slightly resistant (two to four times) to circulin, para-aminosalicylic acid (PAS), and iproniazid. No increase in resistance was noted after growing *M. ranae* in the presence of nisin, patulin, and amithiozone.

The patterns of cross-resistance were tabulated by testing the resistant strains against each toxic agent, allowing the arrangement of the drugs into groups and comparison with previous results obtained using different bacterial species.

The largest group consists of circulin, polymyxin B, and licheniformin (bacterial polypeptides), streptothricin, vinactin, and viomycin (*Actinomyces* polypeptides), catenulin, neomycin, streptomycin, and netropsin. *M. ranae*, however, exhibits within this group a higher degree of selectivity than other microorganisms previously studied. The microbiologic identity of viomycin and vinactin is confirmed. Catenulin and neomycin are closely related but quantitatively different. Hydroxystreptomycin, dihydrostreptomycin, and streptomycin behave identically in these tests.

The second group is composed of chlortetracycline and oxytetracycline.

The third group consists of chloramphenicol, bacitracin, mycomycetin, and PA-89. Penicillin is also partially related to this group by a low degree of cross-resistance. Cross-resistance relationships within this group are of dubious significance because other bacterial species do not reveal similar relationships to any significant degree.

Illudin M and S form a fourth group which exhibits a high degree of cross-resistance.

The fifth group comprises isoniazid and its derivative, iproniazid.

The data do not reveal any other groups of agents related by cross-resistance. Each of the five remaining drugs, including nisin, thiolutin, patulin, PAS, and amithiozone, may therefore be regarded as the sole representative of one of the five remaining groups (six to ten).

Resistant strains of *M. ranae* do not exhibit "collateral sensitivity" (1) to any high degree.

SUMARIO

Estudios Genéticos de la Resistencia Cruzada de los Microbios a los Agentes Tóxicos: III. Resistencia Cruzada del Mycobacterium Ranae a Veintiocho Agentes Antimicobacterianos

Usando la técnica de "placa pendiente" (3), seleccionáronse cepas del *Mycobacterium ranae* casi totalmente resistentes en un solo tiempo a la estreptomicina, la isoniacida y el cloranfenicol; sumamente resistentes a la liqueniformina B, la micomicetina, la viomicina, la vinactina, la neomicina y la catenulina; moderadamente resistentes a la iludina M y la S, la bacitracina, la polimixina B, la estreptotricina, la netropsina, la clortetraciclina (Aureomicina), la oxitetraciclina (Terramicina), la tiolutina, la PA-89 y la penicilina, y ligeramente resistentes (dos a cuatro veces) a la circulina, al ácido para-aminosalicílico (PAS) y a la iproniacida. No se notó aumento de la resistencia después de cultivar el *M. ranae* en presencia de nisina, patulina y tiosemicarbazona nicotinaldehídica.

Los patrones de la resistencia cruzada fueron tabulados comprobando las cepas resistentes con cada agente tóxico, clasificando las drogas en grupos y comparando los resultados con los obtenidos anteriormente con distintas especies bacterianas.

El grupo más numeroso comprende la circulina, la polimixina B y la liqueniformina (polipéptidos bacterianos), la estreptotricina, la vinactina y la viomicina (polipéptidos actinomicéticos), la cateculina, la neomicina, la estreptomicina y la netropsina. Sin embargo, el *M. ranae* manifiesta, en ese grupo, una selectividad mayor que otros microbios estudiados previamente. Queda confirmada la identidad microbiológica de la viomicina y la vinactina. La catenulina y la neomicina son íntimamente afines, pero cuantitativamente distintas. La hidroxiestreptomicina, la dihidroestreptomicina y la estreptomicina se comportaron idénticamente en esos ensayos.

El segundo grupo está compuesto de la clortetraciclina y la oxitetraciclina.

El tercer grupo consta del cloranfenicol, de la bacitracina, la micomicetina y la PA-89. La penicilina está también relacionada parcialmente con este grupo por su poca resistencia cruzada. Las relaciones de resistencia cruzada con este grupo poseen importancia dudosa porque otras especies bacterianas no revelan relaciones semejantes en mayor gado.

La iludina M y la S forman un cuarto grupo que manifiesta un grado alto de resistencia cruzada.

El quinto grupo comprende la isoniacida y su derivado, la iproniacida.

Los datos obtenidos no revelan otros grupos de agentes enlazados por su resistencia cruzada, por lo cual cabe considerar a cada una de las cinco drogas restantes, a saber, la nisina, la tiolutina, la patulina, el PAS y la tiosemicarbazona nicotinaldehídica como único representante de uno de los cinco grupos restantes (seis a diez).

Las cepas resistentes del *M. ranae* no manifiestan en mayor grado "sensibilidad colateral" (*1*).

RESUME

Etudes génétiques sur la résistance croisée bactérienne envers les agents toxiques. III. Résistance croisée du Mycobactérium ranae envers vingt-huit agents antimycobactériens

A l'aide de la technique "gradient plate" (3), des souches de *Mycobactérium ranae* ont été sélectionnée; ces souches montraient dès la génération suivante une résistance presque absolue envers la streptomycine, l'isoniazide et le chloramphénicol; une résistance élevée à la licheniformine B, mycomycétine, viomycine, vinactine, néomycine et caténuline; une résistance modérée à l'illudine M et S, bacitracine, polymyxine B, streptothricine, nétropsine, chlorotétracycline (Auréomycine), oxytétracycline (Terramycine) thiolutine, PA-89 et pénicilline; une résistance faible (deux à quatre générations) à la circuline, à l'acide para-aminosalicylique (PAS) et à l'iproniazide. Aucun accroissement de la résistance n'a été observé après culture du *M. ranae* en présence de nisine, de patuline ou d'aldéhyde nicotinique thiosemicarbazone.

Il a été fait un tableau des aspects de la résistance croisée en soumettant les souches résistantes à des tests utilisant alternativement chacun des agents toxiques, en répartissant

les drogues par groupes, et en comparant les résultats avec ceux précédemment obtenus avec des espèces bactériennes différentes.

Le groupe le plus important comprend la circuline, la polymyxine B et la licheniformine (polypeptides bactériennes), la caténuline, la néomycine, la streptomycine et la nétropsine. Toutefois, parmi ce groupe, le *M. ranae* présente un degré de sélectivité plus élevé que les autres microorganismes précedemment étudiés. L'identité microbiologique de la viomycine et de la vinactine est confirmée. La caténuline et la néomycine sont étroitement apparentées, mais présentent des différences quantitatives. Dans ces tests l'hydrostreptomycine, la dihydrostreptomycine et la streptomycine se comportent de manière identique.

Le deuxième groupe comprend la chlortétracycline et l'oxytétracycline.

Le troisième groupe comprend le chloramphénicol, la bacitracine, la mycomycétine et le PA-89. La pénicilline est aussi partiellement apparentée à ce groupe par un faible degré de résistance croisée. Les rapports de la résistance croisée avec ce groupe n'ont qu'une signification douteuse car des rapports similaires ne sont pas révélés à un degré significatif par d'autres espèces bactériennes.

L'illudine M et S constituent un quatrième groupe qui montre un haut degré de résistance croisée.

Le cinquième groupe comprend l'isoniazide et son dérivé l'iproniazide.

Les données ne révèlent pas d'autres groupes d'agents qui soient apparentés du point de vue résistance croisée. Chacune des cinq autres drogues, nisine, thiolutine, patuline PAS et aldéhyde nicotinique thiosemicarbazone peut donc être considérée comme l'unique représentant de l'un des cinq groupes restants (six à dix générations).

Les souches résistantes du M. ranae ne manifestent la "sensibilité collatérale" qu'à un degré peu important (1).

Acknowledgments

The authors would like to express again their thanks to the donors of the antimicrobial agents used in these investigations. A complete list of sources is contained in the previous papers of this series (1, 2). For use in the study of *M. ranae*, we gratefully acknowledge the additional agents received from G. Reed of Rohm & Haas Company (Triton WR-1339), Dr. W. J. Robbins of the New York Botanical Garden (illudin M and S), and from Dr. J. A. Aeschlimann of Hoffmann-LaRoche (isoniazid and iproniazid).

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A NEW PROCEDURE FOR SCREENING ANTITUBERCULOUS AGENTS[1]

Effect of Chemotherapeutic Agents on Mice Infected with Massive Doses of Tubercle Bacilli Intraperitoneally

JAN ILAVSKY

(Received for publication April 20, 1953)

INTRODUCTION

During the last few years, methods for laboratory evaluation of antituberculous compounds have been devised and, to some extent, standardized. In methods employing mice, the intravenous route of infection is most often used. With this route of inoculation, splenic enlargement develops and macroscopic lesions are seen in the lung (1). The intraperitoneal route of administration was tried by Pierce, Dubos, and Middlebrook (2), who showed that a fatal infection develops but that the minimal infective dose was at least five to ten times that needed when tubercle bacilli are given intravenously. The results of these investigators and those of Donovick (3) indicate that the death rate is more variable following intraperitoneal injection than with intravenous injection. Experiments in this laboratory fully confirm the observations of these investigators. However, it was observed that, following intraperitoneal injection of very large doses of tubercle bacilli, an acute infection was induced which killed mice quickly and consistently. Using this type of infection in mice, a method for evaluating antituberculous agents was elaborated. The present paper describes this method.

MATERIALS AND METHODS

The culture of tubercle bacillus used was a strain of H37Rv obtained September 12, 1950, through the courtesy of William Steenken, Jr., of the Trudeau Laboratory. It has been maintained for more than 100 transfers in Dubos medium (4) with incubation at 37°C. for ten to fourteen days at each culture generation.

The cultures used for infection were grown in 250 ml. Erlenmeyer flasks containing 50 ml. of Dubos medium. These were inoculated with 2 ml. of ten-day-old stock culture and were shaken gently once daily during the course of incubation (5).

Mice of the CF_1 strain (Carworth Farms), weighing approximately 18 gm., were used in all experiments. Upon receipt of a shipment, the mice were observed for forty-eight hours, during which time they gained approximately 1 gm. All of the mice used in any one experiment were of the same shipment. They were placed in groups of 10 in well-bedded stainless steel cages having 110 square inches of floor space. Water and Wayne Fox Food were provided by means of drinking bottles and food bins built into the wire mesh covers. The mice were transferred at least twice a week to fresh, sterilized cages and kept in a room continuously irradiated by ultraviolet light. No evidence of *Salmonella* infection was observed during the studies.

All mice were given 2 ml. of culture intraperitoneally. The mice were restless immediately after the injection, and a few minutes later they were prostrate. During the first twenty-four hours the consumption of food and water was minimal, but increased somewhat by forty-

[1] From the Biological Research Laboratories, Schering Corporation, Bloomfield, New Jersey.

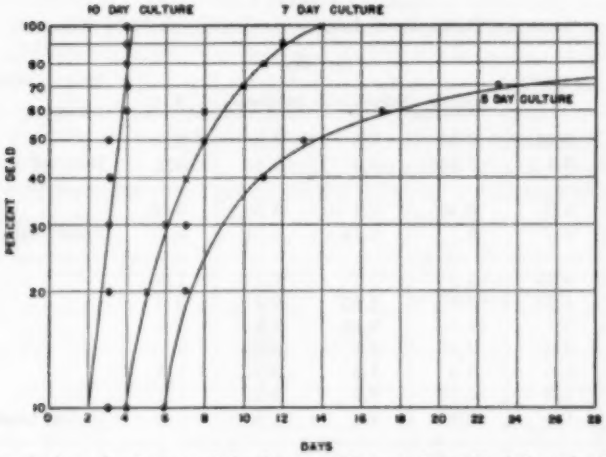

FIG. 1. Age of culture and death rate of intraperitoneally infected mice.

eight hours. A loss of 2.5 to 4 gm. of body weight was regularly observed during this forty-eight-hour period.

Treatment began after forty-eight hours and was continued for seven days. Para-aminosalicylic acid (PAS) or amithiozone was mixed with food which was compressed into pellets and fed *ad libitum*. Isoniazid was dissolved in the drinking water; streptomycin sulfate was injected subcutaneously once a day.

OBSERVATIONS

Effect of age of culture on death rate of mice injected by the intraperitoneal route: Three groups of 10 mice each were injected with 2 ml. of culture intraperitoneally. One group received five-day-old culture; the second, seven-day; and the third, a ten-day-old culture. The results, which are presented in figure 1, showed that the age of the culture influenced the death rate of infected, untreated mice. Those infected with the ten-day-old culture all died within four days, while those infected with the five-day culture died over a relatively long period of time. The death rate of mice injected with seven-day culture was between these two extremes, and cultures of this age were used in all further experiments. The differences in death rates were probably a result of the number of viable bacilli injected. At least, no significant differences in virulence were found when equal doses of equal concentrations of five-, seven- and ten-day-old cultures were tested. It may be noted that the curves shown in figure 1 closely resemble those observed by Donovick following *intravenous injection* of bovine tubercle bacilli (3).

Effect of chemotherapeutic agents in infection induced by the intraperitoneal route: Five groups of 10 mice each were injected with 2 ml. of a seven-day-old culture of H37Rv. After forty-eight hours, treatment was begun. One group received PAS (2 gm. per kg. of food), the second received amithiozone (1 gm. per kg. of food), the third received isoniazid (0.2 mg. per ml. of drinking water), the fourth re-

TABLE 1

AVERAGE DAILY FOOD INTAKE OF SURVIVING MICE (GRAMS)

DAY	CONTROL	TREATMENT				REMARKS
		Isoniazid	Streptomycin	Amithiosone	PAS	
1	2.95	3.05	3.0	3.0	2.9	
2	3.1	3.15	3.1	3.1	3.1	Infected
3	0.5	0.45	0.5	0.5	0.55	
4	0.7	0.7	0.75	0.75	0.7	Treatment started
5	0.75	1.2	1.1	1.1	1.0	
6	1.75	2.8	2.65	2.4	2.0	
7	2.1	3.4	2.85	2.4	2.3	
8	2.0	2.85	2.8	2.45	2.1	
9	1.0	2.9	1.9	2.2	1.6	
10	1.2	3.05	2.6	2.4	1.7	
11	1.5	2.85	2.4	2.4	1.6	End of treatment
12	1.0	2.75	2.1	2.2	1.5	
13	0.8	2.85	2.45	2.4	1.4	
14		3.25	2.35	2.3	1.6	
15		4.1	2.0	2.2	1.5	
16		4.0	2.1	2.3	1.7	
17		3.2	2.4	2.2	1.5	
18		3.6	2.3	2.3	1.6	
19		3.2	2.1	2.3		
20		3.4	2.1	2.4		
21		3.2	2.3	2.2		
22		3.1	2.2	2.5		
23		2.9	2.1	2.4		
24		3.2	2.0	3.0		

All mice dead.

All mice dead.

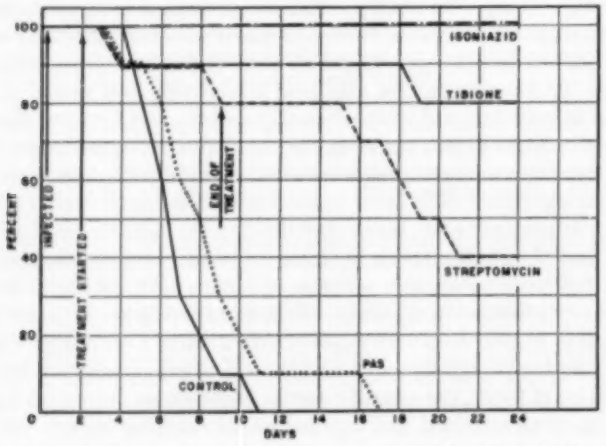

FIG. 2. Survival curves in control mice and mice with various treatments.

ceived streptomycin (1 mg. per mouse) by subcutaneous injection once daily, and the fifth group served as an untreated, infected control. Treatment continued for only seven days. Mortality was recorded daily for twenty-four days, when the surviving mice were killed and autopsied and macroscopic lesions on the surface of the pleura, abdominal wall, and viscera were counted.

Isoniazid treatment gave the best results. All mice of this group ate more food, drank more water, and gained more weight than mice of any other group. All survived, and when sacrificed and autopsied they had a minimum of tuberculous lesions. Amithiozone treatment was less effective than treatment with isoniazid

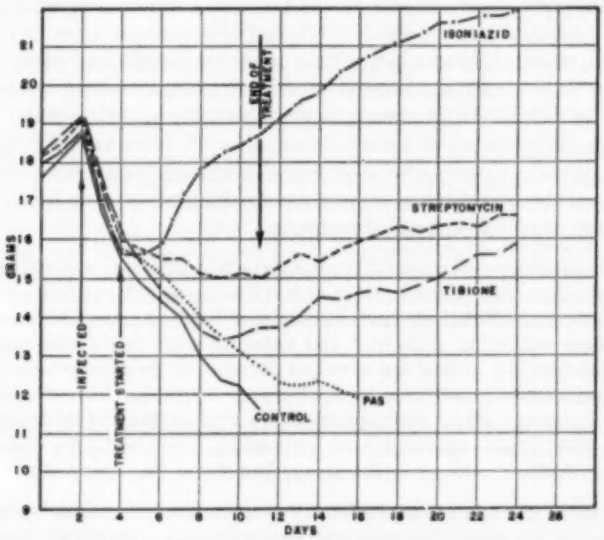

FIG. 3. Average weight curves of control and treated groups of mice.

TABLE 2

VISIBLE LESIONS AT AUTOPSY AS INFLUENCED BY VARIOUS TREATMENTS*

TREATMENT	NUMBER OF MICE USED	DIED	SURVIVED	AVERAGE WEIGHT OF SURVIVING MICE IN GRAMS	MACROSCOPIC LESIONS PER SURVIVING MOUSE†							
					Lung	Thoracic Cavity	Abdominal Wall	Mesentery and Omentum	Liver	Spleen	Kidneys	Total
Untreated control	10	10	0									
PAS	10	10	0									
Streptomycin	10	6	4	16.6	1	3	6.8	6.2	1.8	1.6	0	20.4
Amithiozone	10	2	8	15.9	0	2	3.6	4.8	3.0	1.8	0	15.2
Isoniazid	10	0	10	21.9	0	1.6	2.4	2.1	0	0	0	6.1

* All mice intraperitoneally infected with 2 ml. of a 7-day-old culture of H37Rv grown in Dubos medium. The experiment was terminated 24 days later

† All figures are averages.

but a little better than treatment with streptomycin. PAS treatment was least effective of all. All mice died before the end of the experiment, but the average survival time was a little longer than that of the untreated control group.

The effects of the various treatments on the development of lesions visible at autopsy are shown in table 2.

Data showing the food intake and the survival and weight curves in the various groups are shown in table 1 and in figures 2 and 3, respectively.

COMMENT

Several writers (2, 3) have noted that fatal infections are produced by the intraperitoneal injection of tubercle bacilli, but under the experimental conditions used the mice died after prolonged periods and at inconstant rates. In the present experiments, doses of culture larger than those previously employed have been used. Under these conditions an acute peritonitis and generalized infection occur which can be influenced by known, active chemotherapeutic agents. Since untreated mice die according to a normal frequency distribution curve, the method lends itself to use in screening for antituberculous compounds. Generally, survival time has been the sole criterion of activity. In the experience in this laboratory, the present method has several advantages:

> The treatment period is limited to seven days. The ultimate result can be estimated five or six days after infection. Weak or inactive compounds can be excluded within seven to ten days. Relatively small quantities of drugs are required. Mice with acute tuberculous peritonitis refuse food and water for many hours after infection, and this is reflected in a marked loss of weight. Reversal of the acute process by effective treatment is accompanied by an increase in body weight; i.e., body weight is a useful indicator of drug activity. Further indications of effectiveness of treatment are given by the distribution and number of macroscopic tubercles in the abdominal and thoracic cavities at autopsy on the twenty-first day of the experiment.

Differences in the relative antituberculous effect are discernible by taking into account the weight curve (figure 3) and the lesion count (table 2). As judged by these criteria, isoniazid in the doses used appears to be more effective than streptomycin or amithiozone, and all three substances are more effective than PAS. If the results were judged on the basis of survival only (figure 2), amithiozone would be considered much more effective than streptomycin, but, according to the information revealed by weight changes and the lesion counts, these compounds are in the range of equal effectiveness.

SUMMARY

A mouse test for screening compounds with antituberculous activity is described which has several advantages over those currently used in which prolongation of survival time alone is one criterion. In the present method, massive doses of human tubercle bacilli are injected intraperitoneally in CF_1 mice; treatment is begun after forty-eight hours and continued for seven days.

The experiment is followed for three weeks, during which food consumption, body weight, and mortality are recorded. At the twenty-fourth day, autopsy is

performed on the remaining mice and macroscopic lesions are counted. In an experiment with four drugs of known activity, these criteria gave better assessment of activity than measuring survival time alone. Compounds which have promising activity can be separated from inactive ones after the single week of treatment.

Using this rapid procedure, the activities of PAS, streptomycin, amithiozone, and isoniazid are in accord with those generally found for these compounds.

SUMARIO

Nuevo Procedimiento para la Tria de los Agentes Antituberculosos: Efecto de los Agentes Quimioterapéuticos sobre los Ratones Infectados Intraperitonealmente con Dosis Masivas de Bacilos Tuberculosos

La prueba en el ratón aquí descrita para triar los compuestos dotados de actividad antituberculosa posee varias ventajas sobre las usadas actualmente, en las que la prolongación del tiempo de sobrevivencia constituye una pauta. En el método descrito, se inyectan intraperitonealmente dosis masivas de bacilos tuberculosos humanos en ratones CF_1, y el tratamiento se inicia al cabo de cuarenta y ocho horas, continuando siete días.

El experimento es mantenido durante tres semanas, en las cuales se anotan el consumo de alimento, el peso vivo y la mortalidad. En el vigésimocuatro día, se ejecuta la autopsia en los ratones restantes y se cuentan las lesiones macroscópicas. En el experimento con cuatro drogas, esas pautas proporcionaron una valuación mejor de la actividad que la medición del tiempo de sobrevivencia por sí sola.

Usando ese procedimiento rápido, la actividad del ácido para-aminosalicílico, de la estreptomicina, la amitiozona y la isoniacida convino con la aceptada generalmente para dichos compuestos.

RESUME

Un nouveau procédé pour déceler les agents antituberculeux: Action des agents chimiothérapeutiques sur les souris, après inoculation intrapéritonéale de doses massives de bacilles tuberculeux

Description d'un test chez la souris, révélateur de l'activité antituberculeuse de certains composés, qui présente plusieurs avantages sur les tests couramment utilisés. La prolongation du temps de survie est l'unique critère du test. Dans la méthode actuelle, des doses massives de bacilles tuberculeux humains sont injectés à la souris CF_1 par voie intrapéritonéale: le traitement est commencé quarante-huit heures après et continué pendant sept jours.

L'expérimentation est poursuivie pendant trois semaines, au cours desquelles l'apport alimentaire, le poids du corps et la mortalité sont enregistrés. L'autopsie des souris survivantes est effectuée au vingt-et-quatrième jour et les lésions macroscopiques sont dénombrées. Dans l'expérimentation effectuée avec quatre médicaments connus, ces critères ont permis une évaluation de l'activité des produits, qui est supérieure à la seule détermination du temps de survie. Les composés paraissant doués d'activité peuvent être séparés des composés inactifs après cette semaine de traitement.

Par ce procédé rapide, nous observons que l'activité de l'acide para-aminosalicylique, de la streptomycine, de l'amithiozone et de l'isoniazide correspond à celle généralement attribuée à ces composés.

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Case Reports

INFECTED EMPHYSEMATOUS BULLAE[1]

Report of Five Cases

EMIL ROTHSTEIN[2]

(Received for publication May 28, 1953)

INTRODUCTION

The following case report deals with a rather common and yet largely neglected entity, namely, the development of pulmonary infection in a lung which is the site of emphysematous bullae. The syndrome is fairly easily recognizable, and the present writer has seen ten such cases in recent years. Nevertheless, the literature has few reports of this condition except for single case reports (1, 2) and a previous article (3) dealing with the first five cases of the ten mentioned above. Rigler (4) in *The Chest* describes an instance of nontuberculous infection of a lower lobe emphysematous bulla.

Emphysematous bullae are encountered frequently as chance autopsy findings in older persons (5, 6). Routine roentgenographic surveys often reveal shadows due to such bullae. They appear to occur most frequently in the chests of middle-aged or older men and are likely to be seen at the apex, base, or mediastinal free border of the lung. The shadows of the bullae are commonly bilateral and may or may not be associated with evidences of generalized pulmonary emphysema. The usual roentgenographic appearance is that of an oval area of increased radiolucency containing few or no lung markings. It should be mentioned that lung markings may be seen through a bulla, due to normal lung tissue located anteriorly or posteriorly to the lesion. The size varies from bullae with a diameter of a few centimeters to structures occupying one-half or more of the lung fields. Linear shadows, generally at right angles to the chest wall, may be seen within them, due to interbullous septa. The margins are generally convex, and the walls are very thin, often (especially in the lower lobes) being poorly visualized on the roentgenogram. When bullae rest upon the parietal pleura, the rib shadows usually stand out quite clearly with no visible evidence of pleural reaction.

When infection develops in a lung which has bullae within it, unusual and confusing roentgenographic findings often appear. This occurred in the following cases:

Case 1. P. H., a 27-year-old male, was admitted to the Veterans Administration Center, Dayton, Ohio, on November 21, 1952, because of "nervousness." A chest roentgenogram revealed well-marked emphysematous bullous changes in the left upper lobe and an abnormal shadow in the right upper lobe, characterized by multiloculated air spaces with fluid levels and some surrounding infiltration (figures 1*A* and *B*). The temperature was normal; the leukocyte count was 20,500 per cu. mm. with 76 per cent polymorphonuclears. Penicillin was administered, and the lesion improved roentgenographically. Sputum smears and cultures revealed pneumococci, *Streptococcus viridans*, *Staphylococcus albus*, and *N. catarrhalis*. Numerous sputum smears, sputum cultures, and gastric cultures were negative for *M. tuberculosis*.

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Because of persistent cavity-like shadows, excision of the lesion was performed on January 26, 1953. The gross findings at the time of surgery (Dr. W. G. Eckman) were, in part, "On entering the chest there were multiple emphysematous bullae in all of the segments of the right upper lobe. There were additional emphysematous bullae of the superior segment of the right lower lobe." A right upper lobectomy and a segmental resection of the right lower lobe were performed.

Pathologic examination of the specimen (figure 1*C*), revealed in part:

> "On the anterior aspect of the right upper lobe there are two 1 cm. emphysematous blebs. In the dorsal portion of the lobe there are five localized emphysematous bullae approximately 3 cm. in diameter. The apex of the lobe is characterized by a large thick-walled cavity measuring 9 by 5 cm. On cut section this cavity is divided into irregular spaces by tough tissue bands and by septa. Microscopically, there is marked distortion of the normal architecture with a disproportionate amount of stroma. Scattered throughout there are round, oval, and slitlike cavities. Elsewhere there are marked diffuse fibrosis of the framework and marked thickening of trabeculae. Bronchioles are distorted, irregularly constricted and dilated. Generally, the normal arrangement of alveolar spaces is replaced by large irregular spaces into which project septa of various sizes. The larger irregular spaces are lined by necrotic material."

The diagnoses were: marked bullous emphysema and marked chronic pneumonitis. Numerous smears and cultures from the resected specimen were negative for all bacteria. The patient's recovery was uneventful.

Case 2. C. R., a 47-year-old male, entered the Veterans Administration Center, Dayton, Ohio, on September 20, 1952, with a three-month history of cough, weight loss, and fever. His first chest roentgenogram (figure 2*A*) obtained on September 2, 1952, revealed far advanced cavitary tuberculosis on the left. The upper half of the right lung was the seat of a huge emphysematous bulla, characterized by few lung markings, increased radiolucency, and no evidence of peripheral pleural reaction. Sputum examinations were definitely positive for *M. tuberculosis*.

Three weeks later (figure 2*B*, September 23, 1952) extensive tuberculous changes were present on the right side, with infiltration of the juxta-bullous pulmonary tissue. Subsequent films (figures 2*C* and *D*, October 6, 1952) revealed further changes, including fluid levels in the bullae and pleural thickening about the right upper lobe.

Without the knowledge provided by the film of September 2, 1952, the lesion on the right might quite reasonably have been misinterpreted as a huge tuberculous cavity. Treatment with streptomycin, para-aminosalicylic acid (PAS), and isoniazid resulted in marked improvement (figure 3, April 10, 1953) and marked shrinkage of the bullae, a phenomenon previously observed and reported (3) when tuberculous infection of a bulla developed.

Case 3. A. B., a 53-year-old male, was admitted to the Veterans Administration Center, Dayton, Ohio, on August 28, 1952. Pulmonary tuberculosis had been active in 1942 and again in 1949 but had been considered to be arrested until a recurrence of symptoms in July, 1952. Sputum culture was positive for *M. tuberculosis*. The admission chest roentgenogram (figure 4*A*, September 1, 1952) revealed infiltration and fibrosis in the right upper lobe and a large emphysematous bulla in the right lower lobe. While awaiting the culture reports, the disease progressed in the upper and lower lobes. This was associated with the development of fluid levels in the lower lobe bullae (figures 4*B* and *C*, October 15, 1952). In addition, the bullae decreased markedly in size and, later, could no longer be identified upon subsequent roentgenograms. Streptomycin and PAS were administered with steady roentgenographic clearing.

Case 4. O. S., a 42-year-old male, was admitted to the Veterans Administration Center, Dayton, Ohio, on April 2, 1949. Pulmonary tuberculosis was known to have existed for

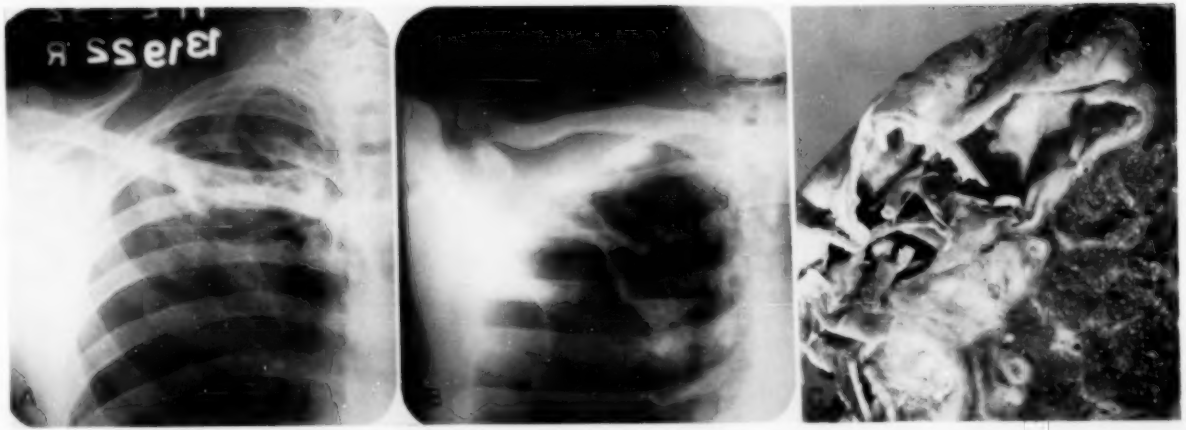

FIG. 1, *Case 1. A* (November 25, 1952). Multiple emphysematous bullae in the right upper lobe. Note the absence of reaction in relation to the pleura adjacent to the first and second ribs. The presence of some pneumonitis is evident in the first and second anterior interspaces.

B (November 28, 1952). A film in lordotic position brings out the fluid level more clearly.

C. Detail of cut section of the right upper lobe.

FIG. 2, *Case 2. A* (September 2, 1952). Far advanced cavitary tuberculosis can be seen in the left lung. The upper half of the right lung is the seat of large bullae. Lung markings are absent and there is complete lack of pleural reaction at the costal margin. The lower walls of the bullae cannot be seen, but there is no evidence of inflammatory reaction.

B (September 23, 1952)). Three weeks later the disease involves the right lung, including the walls of the bullae. Note a fluid level in relationship to the ninth right posterior rib.

C (October 6, 1952). Further extension of the disease has occurred. A fluid level is more apparent. Pleural reaction has occurred over the apex of the bullae, and now extensive bilateral cavitation is suggested.

D (October 6, 1952). Lateral view again illustrates a fluid level.

FIG. 3, *Case 2*. Status after six months of streptomycin, isoniazid, and para-aminosalicylic acid. The resolution of the inflammatory disease is undoubtedly the result of the antimicrobial drugs. The marked shrinkage of the right upper lobe bullae must be attributed to the closure by inflammatory changes of the indirect bronchial or alveolar airways by means of which these bullae had remained aerated prior to the onset of the tuberculosis.

two years. A roentgenogram obtained on admission to the hospital (figure 5*A*, April 5, 1949) revealed extensive tuberculosis in the right upper lobe and extensive emphysematous bullae in the right lower lobe. Subsequent roentgenograms (figures 5*B* and *C*, July 21, 1949) showed a decrease in the size of the right lower lobe bullae, associated with a fluid level and thickening of the bullous walls. As noted in other cases, this was followed by marked shrinkage of the bullae (figure 5*D*, January 20, 1950) and their eventual disappearance.

Case 5. K. H., a 43-year-old male, was admitted to the Veterans Administration Center, Dayton, Ohio, on February 12, 1953. Dyspnea upon exertion had been present from 1945, gradually becoming more marked, to the point of producing complete disability. Cough, with no sputum, was present for three years. During the past year there had been chronic productive cough with chest pain. Chest roentgenograms obtained in 1947 revealed well-marked evidence of emphysema at both bases and in both upper lobes. No evidence of infection could be visualized on these films.

In May 1952, considerable increase in the emphysema was noted. Both lower lobes, but especially the right, were the seat of large emphysematous bullae, as were both upper lobes. Bilateral infiltration, fibrosis, and pleural reaction were visualized. Numerous sputum examinations (at Clark County Sanatorium, Springfield, Ohio) were negative for *M. tuberculosis*.

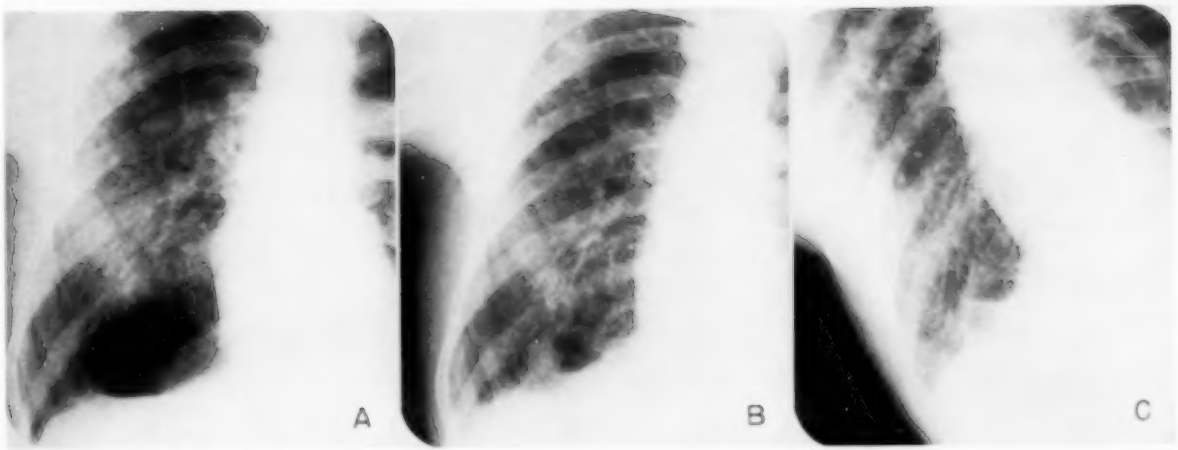

FIG. 4, *Case 3. A* (September 1, 1952). Scattered infiltration can be seen in the right lung. A large bulla occupies a portion of the right lower lobe.

B (October 15, 1952). Some increase in the disease has occurred. Concomitantly (and presumably as a result of tuberculous involvement of the bullous walls), there is noted marked shrinkage of the bulla plus thickening of the wall and two fluid levels.

C (October 15, 1952). The patient is tilted to the right, to illustrate the fluid level more clearly.

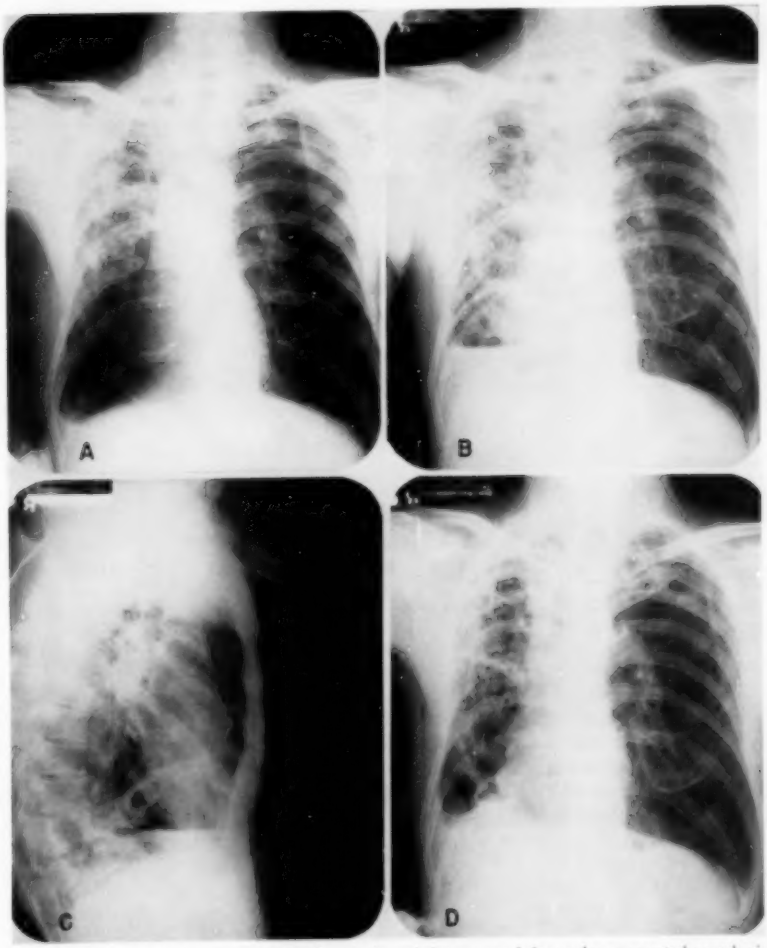

FIG. 5, *Case 4*. *A* (April 5, 1949). Extensive right upper lobe pulmonary tuberculosis is noted with a large bulla at the base of the lung.

B (July 21, 1949). Presumably as a result of tuberculous involvement of the bullous walls, these have become much more prominent and a fluid level has developed.

C (July 21, 1949). Lateral film again illustrating the bulla with fluid level.

D (January 20, 1950). Marked shrinkage has occurred. A fluid level can still be seen.

At the time of the present admission, the patient appeared chronically ill. The temperature was normal. The leukocyte count was 9,100 per cu. mm. with 79 per cent polymorphonuclears. Sputum examinations revealed gram-positive cocci and diphtheroids. *Staphylococcus albus* and pneumococci were recovered on culture. No acid-fast bacilli or fungi were recovered by concentration or culture. Although the chest roentgenogram (figure 6, February 15, 1953) suggested pulmonary tuberculosis with extensive cavitation, the final diagnosis was emphysematous bullae with infection.

FIG. 6, *Case 5*. Extensive bilateral emphysema with bullae can be visualized. The infected upper lobe bullae mimic pulmonary tuberculosis.

COMMENT

The etiology of emphysematous bullae is unknown. Although many persons in whom bullae are found have a history of chronic bronchitis or asthma, this is not always the case. Symptoms and roentgenographic findings of chronic infection are generally absent and, when lobes showing emphysematous bullae are removed surgically or at autopsy, no other pulmonary disease is characteristically associated with them (5, 6, 7). Pathologically (5)

> . . . the air vesicles are much distended and their walls proportionately thinned. Rupture of their walls has often resulted in several alveoli being merged to form large irregular spaces, and the remains of ruptured septa may project into these for a longer or shorter distance. The interlobular septa resemble the alveolar walls in that they also are much thinned.

Bullae are distinguished from blebs in that a bleb is subpleural only, whereas a bulla lies within the pulmonary tissue proper. No accurate distinction can be made without actual inspection of the involved lung. Bullae are distinguished from cysts by the fact that the latter are probably congenital in origin and are lined by cuboidal or columnar epithelium (6). Cysts frequently are filled partially or completely with fluid produced by the lining epithelium; in the absence of infection, fluid is rarely, if ever, present within a bulla. In addition, cysts often have frank large bronchial communications whereas, in most instances, bullae have indirect communications with the bronchial tree. This is shown by their usual failure to collapse readily at autopsy (5) or at the time of thoracotomy (8), by the difficulty encountered in filling them with iodized oil by means of bronchography, by the results of physiologic tests which commonly reveal air trapping (9), and by the infrequency of superimposed infection except when the adjacent lung is involved.

When a lobe which is the seat of emphysematous bullae develops an infection, this may involve the bullous walls and septa and by so doing produce an unusual and characteristic roentgenographic picture. In 8 of the 10 cases observed by the present writer, the infection was tuberculous. The chief features are an extension of infection along the interbullous septa, the development of fluid levels within the bullae, and the gradual obliteration of the bullae with absorption of their contents. Roentgenographically, these lesions may then suggest multiple extensive cavitation or abscess formation. Certain aspects of the complete picture may be of value in making a differential diagnosis:

Knowledge of pre-existing bullous changes in the involved lung
The presence of obvious bullae in other areas of the same lung or in the other lung
The very rapid appearance of fluid levels and extensive apparent cavitation after only a few days' illness
The relatively slight involvement of the surrounding lung
The absence, at the start at least, of any pleural reaction
The generally benign clinical picture

These features are not usually present in cases demonstrating large lung abscesses, large tuberculous cavities, or infected cysts, particularly before antimicrobial therapy has been given.

In the 8 tuberculous cases observed, the development of inflammatory changes and fluid levels in the bullae was asymptomatic. Fever, chills, and increased expectoration were not observed. In no instance could this development be interpreted as one of necessarily unfavorable prognosis except that in each case it gave further evidence of the activity of the tuberculous process. The usual course of events consisted in each case of gradual shrinkage of the bullae with absorption of the air and fluid and eventual replacement of the previous shadows by nondescript linear or irregular densities interpreted as "fibrosis." This was observed without, as well as with, the use of antituberculous chemotherapy. Infection of a pulmonary cyst does not generally result in its disappearance, perhaps due to the persistence of the epithelial lining which prevents obliteration and necessitates surgical removal. In no instance has the opportunity presented itself for examination of a tuberculous lobe at any time in the course of an infection of a bulla.

Proved nontuberculous infection of bullae has been observed on two occasions (*Cases 1* and *5*). Other cases have been seen which suggested this syndrome, but the inability to prove them by resection or by the demonstration on earlier roentgenograms of bullae in the involved area has prevented their inclusion in this study. Both patients responded fairly well to antimicrobial therapy, and one was subsequently cured by resection of the involved portion of lung.

In the 8 tuberculous cases, no special management seemed necessary. No new symptoms needing treatment appeared concomitantly with fluid levels in the bullae. All resolved spontaneously with obliteration of the bullae after a period of bed rest, which in 2 of the most recent cases was associated with chemotherapy. Neither surgical drainage nor excision seemed to be indicated at any time. A nontuberculous infection of a bulla should probably be treated with suitable antimicrobials until maximum resolution has occurred, but experience has not been adequate to indicate whether eventual surgical intervention is necessary.

SUMMARY

Five cases are reported of pulmonary infection superimposed upon previously existing emphysematous bullae. Three of these infections were tuberculous. The characteristic clinical and roentgenographic features of the syndrome are described. The resemblance of

infected bullae with fluid levels to tuberculous cavities and lung abscesses makes recognition of this syndrome of particular significance.

SUMARIO

Bullas Enfisematosas Infectadas

Preséntanse 5 casos de infección pulmonar sobrepuesta a previas flictenas enfisematosas. Tres de esas infecciones fueron tuberculosas. Descríbense las típicas características clínicas y radiográficas de dicho síndrome. La semejanza de las bullas infectadas que muestran niveles de líquido a las cavernas tuberculosas y a los abscesos pulmonares imparte importancia particular al reconocimiento de ese síndrome.

RESUMÉ

Bulles d'emphysème infectées

L'auteur rapporte cinq cas d'infection pulmonaire greffée sur des bulles d'emphysème. Trois de ces infections étaient tuberculeuses. Les caractéristiques cliniques et les aspects radiographiques de ce syndrome sont décrits. La ressemblance entre ces bulles infectées, avec présence de liquide, et les cavernes et abcès du poumon revêt une importance particulière dans le diagnostic de ce syndrome.

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HEPATOLYSIS[1,2]

An Operation for the Division of Adhesions Between Diaphragm and Liver in the Course of Pneumoperitoneum Treatment

MITSUNORI NARITA

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No definite conclusion has yet been reached to account for the effectiveness of pneumoperitoneum therapy. However, it is very widely recognized that elevation of the diaphragm is the most important direct factor. The desired elevation of the diaphragm may be impaired or prevented by adhesions between the diaphragm and the liver which result from previous pleurisy or peritonitis. The frequency of complete or almost complete adhesion between diaphragm and liver on roentgenograms, as reported by Yamamoto, is 6.8 per cent of all cases of pneumoperitoneum. On this service, such diaphragmatic adhesions were encountered in 2 of 31 patients who received pneumoperitoneum.

Accordingly, a surgical procedure has been devised to eliminate the mechanical obstruction in cases in which insufficient elevation of the diaphragm resulted from pneumoperitoneum. The operation which is described in the present report consists of dividing the restraining adhesions. The operation is a laparotomy, with an incision which follows the right costal margin.

The following report of a case in which this operation was used will serve to illustrate the procedure employed.

CASE REPORT

The patient was a young man, 19 years of age, whose tuberculin test had become positive five years previously. He had a history of pleurisy on the right and left sides at 17 and at 18 years of age, respectively. He was admitted to this hospital on September 7, 1950, with an exudative, cavernous lesion of the right lower lobe, frequent hemoptysis, and with *M. tuberculosis* consistently demonstrable on culture of sputum.

Right phrenic nerve exeresis was performed on November 27, 1950 (figure 1). Pneumoperitoneum was induced on March 4, 1951, after the administration of 28 gm. of streptomycin. Thereafter, hemoptysis and bloody sputum disappeared, the sputum was no longer positive for *M. tuberculosis*, and the patient's condition was definitely improved.

Nevertheless, a roentgenogram obtained after the institution of pneumoperitoneum revealed a diffuse shadow between the liver and the diaphragm, evidently as a result of adhesions. After seven months of observation, there was no tendency for the adhesions to divide spontaneously (figure 2). Because a greater collapse was advisable in view of the position and character of the tuberculous lesion, hepatolysis was performed on October 24, 1951.

Operation: Under percamine spinal anesthesia the upper abdominal cavity was reached through a right costal margin incision. Various sizes of restiform and membranous adhesions were found between the thickened diaphragm and over the whole surface of the right lobe of the liver to the right of the falciform ligament. The adhesions extended posteriorly to the site of physiologic adhesion between the liver and the diaphragm (the bare area). The hepatic capsule was thickened and opaque. It was obvious that spontaneous division of the adhesions encountered could not be expected. They were divided at operation as far as the

[1] From the National Nagano Sanatorium and the Jikeikai Medical College, Tokyo, Japan.

[2] Presented before the 49th annual meeting of the Japanese Society of Internal Medicine, Osaka, Japan, April 1-3, 1952.

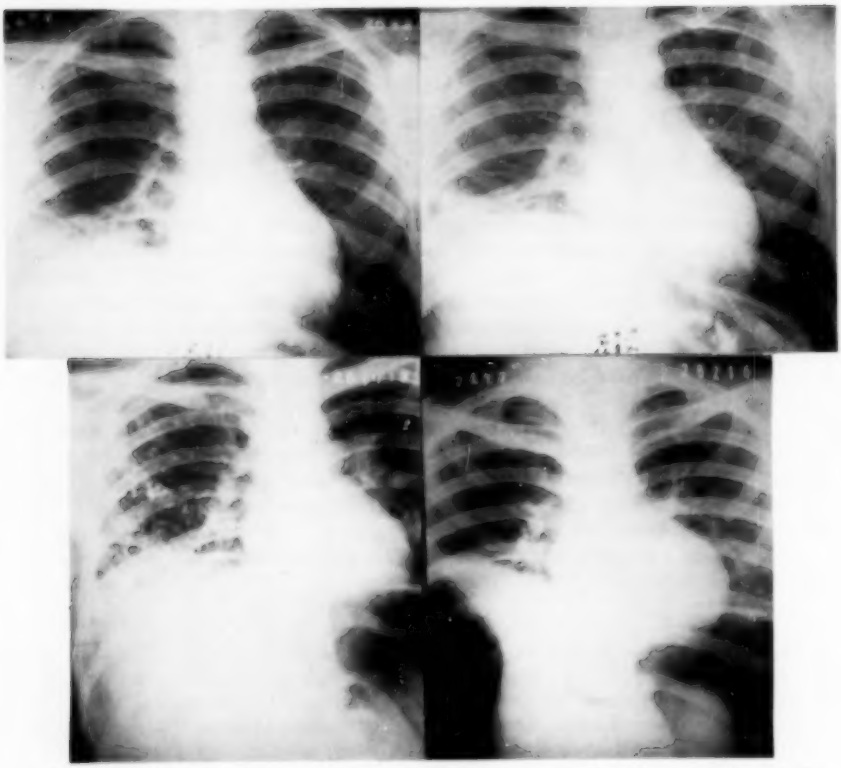

FIG. 1. (Upper left) Roentgenogram obtained March 2, 1951, after exeresis was performed.

FIG. 2. (Upper right) Roentgenogram obtained April 13, 1951, after institution of pneumoperitoneum, which revealed a diffuse shadow between liver and diaphragm.

FIG. 3. (Lower left) Roentgenogram obtained on November 13, 1951, after hepatolysis, showing residual contrast.

FIG. 4. (Lower right) Roentgenogram obtained on February 16, 1953, showing re-formation of adhesions one year and four months after hepatolysis.

coronal ligament. The division was performed mostly by careful manual stripping but partly with scissors between ligatures. When the division had been completed, the wound was closed in two layers without drainage.

The loss of blood was very slight. During division of the adhesions, the patient complained of shoulder pain, first on the right, later on both sides; the pain was evidently reflex in origin. No signs of shock were noted. Soon after the operation, approximately 800 cc. of air were injected into the abdominal cavity to prevent fresh adherence. The upper part of the body was raised after the termination of anesthesia. Refills of air were given frequently following the disappearance of postoperative meteorism.

COMMENT

It was found roentgenographically after hepatolysis that the right diaphragm was 1.5 cm. higher than its preoperative height, with a corresponding increase in the radio-trans-

parent area indicating the presence of air below the diaphragm (figure 3). Thus, the attempt to eliminate the mechanical restraint due to traction between the diaphragm and the liver, the purpose of this operation, was essentially accomplished. At present, one year and four months after hepatolysis, repeated examinations have failed to reveal any side effects such as mobility of the liver or disturbance of liver function. The pulmonary lesion has improved, and the general condition is very good.

Two months after operation, however, there was seen on the roentgenogram a shadow which suggested a membranous re-adhesion in the anterior portion of the space between the liver and the diaphragm. The patient had shown a tendency to accumulate fluid even in the preoperative period. It is believed that the fluid, together with the consequent decreased air refills customary in such situations, accounted for the formation of adhesions. In spite of this, the diaphragm has remained at the same, or even at a slightly higher, level than in the immediate postoperative period. Observation of this re-formation of adhesions is being continued (figure 4).

As mentioned above, a greater degree of collapse has resulted from the hepatolysis, but it has not come up to expectation. One reason for this is considered to be the greater rigidity of the diaphragm due to its increased thickness following pleurisy. Some thought has been given to means of increasing the surface area of the diaphragm to permit it to rise higher.

Many observations remain to be made in the future. Among these may be mentioned the influence of hepatolysis on all abdominal organs, especially the liver; indications for operation; and, incidentally, the effects on the physiology of the cessation of pneumoperitoneum.

In the writer's experience, it is not unusual to note on the roentgenogram a uniformly diffuse shadow suggesting adhesion below the diaphragm which, in one to four months, is replaced by the radio-transparent layer of air seen on the usual pneumoperitoneum roentgenogram. In such situations, there are either no roentgenographically demonstrable adhesions or only some cordlike shadows remaining. Surgery is obviously not needed in such cases, and hepatolysis for adhesions should be considered only in patients in whom these shadows have persisted for five months or longer. It is also believed to be unnecessary to operate on patients with a few restiform adhesions only.

The writer believes that there is room for improvement in the operative method and for determining the best type of anesthesia; there is also the problem of decreasing the degree of meteorism encountered postoperatively. The etiology of re-adhesion and its significance in pneumoperitoneum also require further study.

Acknowledgment

The writer wishes to thank Dr. R. K. Start and Prof. N. Hayashi for their interest and encouragement in this work and in the preparation of the manuscript.

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Notes

A PURIFIED TUBERCULIN FRACTION FROM UNHEATED CULTURES IN TESTING BCG-VACCINATED SUBJECTS

A Preliminary Study

In a previous report,[1] data were presented which supported the opinion that a partly purified tuberculin fraction may be a more reliable indicator of tuberculin sensitivity, especially in BCG-vaccinated persons, than is the whole mixture of active proteins. The most potent fraction of the culture filtrates apparently includes several different proteins,[2] and it seems unlikely that a practical standard for large-scale use can be made containing only a single pure protein. It should be possible, however, to prepare a standard consisting of a protein mixture "purified" in the sense that it is substantially free of inactive material and of the relatively undesirable C group of proteins. In order to devise satisfactorily reproducible methods for even such a partial purification, it is necessary to work with native undenatured protein. The present preliminary report describes a study of a partially purified tuberculin from unheated culture filtrates.

Method of preparation: Cultures of a human strain of *Mycobacterium tuberculosis*, No. 48189, were grown on Long's medium for eight weeks and harvested on a large Seitz filter. The filtrates were dialyzed under toluene for five to seven days against running tap water at approximately 10°C., then dried from the frozen state. The dried material was redissolved at one-fiftieth the volume of the original filtrate and the solution was acidified to pH 4 with acetic acid. The precipitate, consisting of the C protein described by Seibert[2b] was washed once with acetic acid at pH 4.

In this and all subsequent steps the protein solutions were held at temperatures of 5°C. or lower. All centrifugations were carried out in a refrigerated centrifuge. Precipitation with alcohol was carried out in a room maintained at approximately 0°C., pre-cooled alcohol being added slowly to the cold protein solutions with constant stirring.

The fraction soluble at pH 4 was neutralized, dialyzed overnight, and lyophilized. The dried material was redissolved in 0.02 M sodium acetate at one-fiftieth the volume of the original culture filtrate, the pH adjusted to approximately 6.8, and the solution mixed with one-half its volume of alcohol. The precipitate, which consists chiefly of polysaccharide II,[2b] was centrifuged off. To the supernatant was added sufficient zinc acetate to give a concentration of 0.01 M, based on the volume of the original aqueous solution, and alcohol to bring the final alcohol concentration to 50 per cent. The precipitate so formed contains the desired tuberculin proteins, while the material in the alcoholic supernatant consists largely of polysaccharides and nonprotein nitrogen compounds.

The zinc-protein precipitate was dialyzed overnight, and the resulting solution was acidified to pH 4 and mixed with 5 per cent of its volume of alcohol. A small precipitate of C protein was separated and discarded, and the supernatant was neutralized and dialyzed overnight. The successive steps, (*1*) precipitation by 0.01 M zinc from 50 per cent alcohol and (*2*) acidification at pH 4 with 5 per cent alcohol, were then repeated. The solution so obtained was freed from zinc by filtration through a column of "Amberlite" IRC-50 ad-

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justed to approximately pH 9, neutralized, dialyzed against several changes of distilled water, and sterilized by filtration through a Mandler candle. The protein concentration was estimated from the nitrogen determined by the Kjeldahl method. The zinc content of a solution containing 2 mg. of protein per ml. was less than 2 parts per million.[2]

The material so prepared was designated Fraction T(NY) No. 1–53. The yield was approximately 120 mg. of protein per liter of culture filtrate. For use in skin tests the protein was diluted in sterile phosphate buffer at pH 7.3 to contain per 0.1 ml., respectively, 0.00002 mg. (1 tuberculin unit), 0.0002 mg. (10 tuberculin units), and 0.005 mg. (100 tuberculin units) of Fraction T(NY). Fresh dilutions were made before use and discarded the same day.

Care was taken to use new 1 ml. tuberculin syringes and 26-gauge intradermal point needles, kept separate for each type and dilution of tuberculin throughout the study. Special attention was given to remove residual tuberculin in syringes and needles after each testing session by leaving them in 45 per cent urea during twenty-four hours, followed by overnight rinsing in running water when they were dried and autoclaved.

The midvolar surface of the left forearm was used for the first-strength doses and, if there was no reaction forty-eight hours later, the same surface of the right forearm was used for injection of the intermediate-strength doses. Non-reactors were finally given the second-strength doses on the left forearm at approximately 5 inches' distance from the first-strength dose sites.

Interpretation of the tests was made at forty-eight hours, and a positive reaction required definite induration or edema measuring at least 6 by 6 mm. in diameter and 3 mm. thick. Thickness was estimated by folding the skin between caliper prongs. One-half of the thickness multiplied by the two maximum diameters of the indurated area (for example: 1.5 by 6 by 6 mm. = 54 mm.^{3}) provided an estimate of the volume of induration. Erythema alone was disregarded.

The subjects consisted of 108 unvaccinated healthy persons, without history of tuberculosis, from 18 to 60 years of age and 52 student and graduate nurses from 18 to 35 years of age who had been vaccinated with BCG by the multiple-puncture technique two to fourteen months previously.

In order to minimize variations in preparing and administering the tuberculins and in interpreting the skin reactions, the work was done by one person.

In table 1 are summarized the reactions to first-, intermediate-, and second-strength doses of Old Tuberculin (OT), lot 61A (expiration date November 27, 1954, prepared and standardized by the Division), PPD (Parke, Davis and Company, lot 015089-X first strength, expiration date September 22, 1955), and lot 019715-K second strength (expiration date February 25, 1956), and Fraction T(NY). It will be noted that a striking equivalence of positive reactions to 1, 10, and 100 tuberculin units of OT and Fraction T(NY) was obtained among the healthy controls and the BCG-vaccinated persons and that the positive reactions to similar doses of PPD averaged 8.23 per cent less among healthy controls and 13.36 per cent less among BCG-vaccinated subjects. It is especially noticeable that OT and Fraction T(NY) elicited significantly greater positive reactions to 1 tuberculin unit than did PPD in the BCG-vaccinated persons. The difference would suggest that Fraction T(NY) more accurately determines the incidence and persistence of allergy among BCG-vaccinated persons than does PPD. The data in table 2 indicate that Fraction T(NY) elicited a greater and more sharply demarcated area of induration or edema than either OT or PPD. This was especially true of the first-strength dose.

[2] For the zinc determination the authors are indebted to Mr. James O'Brien.

TABLE 1

COMPARISON OF POSITIVE TUBERCULIN REACTIONS 48 HOURS AFTER INTRACUTANEOUS INOCULATIONS OF FIRST-, INTERMEDIATE-, AND SECOND-STRENGTH DOSES OF 0.01, 0.1, AND 1.0 MG. OF OT AND 0.00002, 0.0002, AND 0.005 MG. OF PPD AND FRACTION T(NY)

GROUP	TUBERCULIN	FIRST-STRENGTH			INTERMEDIATE			SECOND-STRENGTH			TOTAL	
		Number Tested	Positive		Number Tested	Positive		Number Tested	Positive		Positives	
			Number	Per Cent		Number	Per Cent		Number	Per Cent	Number	Per Cent
Control	OT	108	68	62.9	40	25	62.5	15	1	6.6	94	87.03
	PPD	108	60	55.5	48	21	43.7	27	4	14.8	85	78.70
	T(NY)	108	68	62.9	40	25	62.5	15	1	6.6	94	87.03
BCG	OT	52	26	50.0	26	24	92.3	2	1	50.0	51	98.07
	PPD	52	15	28.8	37	25	67.6	12	4	33.3	44	84.61
	T(NY)	52	26	50.0	26	24	92.3	2	1	50.0	51	98.07

TABLE 2

COMPARISON OF VOLUME OF INDURATION (MM.^{3}) OF POSITIVE TUBERCULIN REACTIONS ($\geq 6 \times 6 \times 1.5$ MM. = 54 MM.^{3}) 48 HOURS AFTER INTRACUTANEOUS INOCULATIONS OF FIRST-, INTERMEDIATE-, AND SECOND-STRENGTH DOSES OF OT, PPD, AND FRACTION T(NY)

GROUP	TUBERCULIN	FIRST-STRENGTH		INTERMEDIATE		SECOND-STRENGTH	
		Number Positive	Induration (Mm.^{3})	Number Positive	Induration (Mm.^{3})	Number Positive	Induration (Mm.^{3})
Control	OT	68	2,194 ± 2,439*	25	513 ± 727*	1	1,125
	PPD	60	604 ± 819	21	202 ± 156	4	603 ± 211
	T(NY)	68	3,853 ± 6,572*	25	854 ± 494	1	1,143
BCG	OT	26	381 ± 444*	24	364 ± 192*	1	821
	PPD	15	253 ± 150	25	197 ± 94	4	705 ± 115
	T(NY)	26	630 ± 210*	24	516 ± 223*	1	1,922

* The difference between these figures and those for PPD is significant by

$$t = \frac{x - x'}{s}\sqrt{\frac{(n_1 + 1)(n_2 + 1)}{n_1 + n_2 + 2}}$$

at the $P = 0.01$ level.

Standard deviation (±): $\sigma = \sqrt{\frac{S(d^2)}{n - 1}}$.

Confirmation of our experience with Fraction T(NY) has been given us by Dr. J. D. Aronson of the Henry Phipps Institute in Philadelphia, who wrote[4] that:

> . . . In one of our last studies we compared the sensitivity of PPD obtained direct from the United States Public Health Service with your Fraction T(NY) among the unvaccinated general population. Of the 164 persons tested 53 per cent reacted to

[4] ARONSON, J. D.: Personal communications, May 8 and June 18, 1953.

0.00002 mg. PPD and 61 per cent reacted to 0.00002 mg. Fraction T(NY). The reaction to Fraction T(NY) was significantly stronger and more definite than with PPD. . . . My impression is that we get a better and more sharply defined edema with your tuberculin than we get with PPD supplied us by Doctor Seibert, and certainly better than with the 'Weybridge PPD'. . . . The local reaction to both Fraction PT(NY)[1] and Fraction T(NY) was comparable. In general the reactions to your tuberculins were better than to our OT and even after 96 hours your tuberculin stood out more definitely than OT.

These results suggest that a partly purified tuberculin of satisfactory specificity and high potency can be rather easily prepared from unheated filtrates by precipitation with zinc acetate and alcohol. The method as given is not wholly satisfactory, notably in that removal of C protein is incomplete. Efforts to improve the procedure are continuing.

In summary: A comparison has been made of the specificity and potency of OT, PPD, and a partly purified tuberculin Fraction T(NY) in healthy controls and in BCG-vaccinated subjects. Equivalence in positive reactions obtains between OT and Fraction T(NY), while PPD gave somewhat fewer positive reactions in healthy controls and significantly less in BCG-vaccinated subjects than either OT or Fraction T(NY). The local reaction to one tuberculin unit of Fraction T(NY) (0.00002 mg.) is greater and more sharply defined than to the equivalent dose of PPD or OT. Fraction T(NY) tuberculin would appear to be a more sensitive indicator of tuberculin sensitivity than PPD, especially in BCG-vaccinated subjects.

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Mary C. Pangborn
Konrad Birkhaug

July 27, 1953

EXPERIMENTS WITH A NEW CULTURE METHOD FOR TUBERCLE BACILLI

Some preliminary results which were obtained with a method of digestion and decontamination of sputum for the isolation of *Mycobacterium tuberculosis* are presented. The method involves the combined use of pancreatic enzymes and quaternary ammonium compounds. The latter, as is well known, have a comparatively weak effect on tubercle bacilli in contrast to their effect on most other bacteria.

A pancreatic preparation produced by Novo (Copenhagen) was found suitable for digestion. A 2 per cent (w/v) aqueous solution of the pancreatin was centrifuged and filtered. To the filtrate was added an equal volume of a 2 per cent solution of Desogen (Geigy, Basel). The fluid product of Desogen (methylphenyl-dodecyl-trimethyl-ammonium-methosulphate) appeared better fitted for the purpose than the one distributed in substance ((dodecanoyl-N′-methyl-aminoaethyl)-(phenyl-carbamyl-methyl)-dimethyl-ammoniumchloride) and was used in this investigation. The commercial Desogen solution contains a small addition of perfume, but a pure product was obtained by courtesy of the firm. The pancreatin-Desogen solution (PD) was stored at room temperature.

To a portion of the sputum was added an equal volume of PD, and the mixture was kept in 13 mm. test tubes on the desk (20° to 24°C.) for four hours in some experiments and for twenty-four hours in others. The temperature during the night varied from 16° to 20°C. Then the liquefied contents were sucked up in Pasteur pipettes and blown out several times, after which one drop of the material was seeded onto each of 4 Löwenstein-Jensen slants.

Parallel experiments were run with the sodium hydroxide method (NaOH), which is the routine method in this laboratory. In an extensive work[1,2] recently, NaOH was found superior to three other methods of homogenization and decontamination.

The same volume of sputum was employed with the NaOH method as with the PD method. After addition of 2 volumes of 4 per cent NaOH, the tubes were incubated at 37°C. for twenty minutes, centrifuged for twenty minutes, the sediments were neutralized with N HCl, and 3 drops of each sediment were inoculated on each of 4 Löwenstein-Jensen slants.

Since the neutralization and inoculation of the samples takes some time, there was a difference in the length of NaOH treatment of samples handled at the same time, amounting to a maximum of thirty to forty minutes at room temperature when many samples were handled. A similar, but smaller, time variation occurred in the case of the PD method.

The inoculated Löwenstein-Jensen slants were incubated for six weeks.

The size of the inoculum was slightly in favor of the NaOH method, representing 10 per cent more sputum than in the PD method. Furthermore, centrifugation of the NaOH-treated sputum would tend to concentrate the tubercle bacilli in the sediments.[3]

The results of the comparison of the two methods with respect to yield of positive results are seen in table 1. The difference in favor of the PD methods may be considered statistically significant. Probably there is a positive correlation between the two methods since a number of specimens contain so many tubercle bacilli that they are sure to give

[1] Monthly Bulletin, Ministry of Health Service, 1952, *11*, 187.

[2] Lancet (editorial), 1952, *2*, 723.

[3] Klein, G. C., Maltz, M., Cummings, M. M., and Fish, C. H.: Am. J. Clin. Path., 1952, *22*, 581.

TABLE 1

CULTIVATION OF M. TUBERCULOSIS FROM SPUTUM AFTER TREATMENT WITH PANCREATIN-DESOGEN (PD) AND WITH NaOH

Incubation Time with Pancreatin-Desogen for Four Hours (661 Samples) or for Twenty-Four Hours (230 Samples)

HOURS AT ROOM TEMPERATURE WITH PD	SPECIMENS POSITIVE FOR M. TUBERCULOSIS				DIFFERENCE BETWEEN RELATIVE FREQUENCIES: $\Delta = p_{PD} - p_{NaOH}$	STANDARD ERROR OF THE DIFFERENCE σ_Δ	$K = \frac{\Delta^*}{\sigma_\Delta}$
	Total	Both methods (a)	PD only (b)	NaOH only (c)			
4	167	110	39	18	0.121	0.0473	2.56
24	63	31	26	6	0.294	0.0896	3.28

* The following formulas were used in calculating the standard error of the difference and the quotient K which is the difference between the relative frequencies measured in the standard error units:

$$\sigma = \sqrt{\frac{p \cdot q}{n}}, \quad n_{PD} = a + b, \quad p_{PD} = \frac{b}{a+b}, \quad q_{PD} = 1 - p_{PD}, \quad n_{NaOH} = a + c,$$

$$p_{NaOH} = \frac{c}{a+c}, \quad q_{NaOH} = 1 - p_{NaOH}, \quad \sigma_\Delta = \sqrt{\sigma^2_{PD} + \sigma^2_{NaOH}}.$$

TABLE 2

FREQUENCY OF CONTAMINATION WITH TWO DIFFERENT HOMOGENIZATION PROCEDURES

METHOD		NUMBER OF SLANTS		DIFFERENCE: $\Delta = a - b$, RESP. $c - d$	STANDARD ERROR OF THE DIFFERENCE σ_Δ	$\frac{\Delta}{\sigma_\Delta}$
		Total	Contaminated Slants			
PD, 4 hours, room temperature	(a)	2,644	469	74	26.87	2.75
NaOH	(b)	2,644	395			
PD, 24 hours, room temperature	(c)	920	126	−33	15.50	2.13
NaOH	(d)	920	159			

positive results by both methods. If that is so, the true standard errors of the differences will be smaller than those computed. This would mean that the differences are actually more highly significant than is shown by the computations used.

Furthermore, the growth of tubercle bacilli seemed to occur earlier when the PD method was used.

In table 2 it may be seen that the PD method used at room temperature for twenty-four hours gave fewer contaminated slants than did the NaOH method, a difference which is near to a level of significance. On the other hand, it seems that the lower contamination rate with the NaOH method compared with the PD method used for four

hours is statistically significant. However, with the PD method used for twenty-four hours and for four hours, 6.74 per cent and 5.84 per cent, respectively, of the slants showed minor or larger erosions of the surface without any visible contamination, probably due to enzymatic digestion. The erosions did not appear to interfere with the growth of the tubercle bacilli or the reading of the results.

The favorable results of the PD method used for twenty-four hours at room temperature suggest the possibility of addition of the digestor at the place of collection of the sputum, enabling the laboratories to make direct inoculations from the received specimen containers. Experiments are conducted along such lines.

Experiments are also carried out at different temperatures and by using buffered solutions of pH 8 to 9, near the optimum for the pancreatic digestion. It is well known that many quaternary ammonium compounds have a greater germicidal effect at an alkaline than at a neutral reaction.

Some of the more viscous sputum specimens were not quite homogenized by the PD method, especially after four hours. The sucking through a Pasteur pipette prior to the inoculations, however, broke down clumpy material.

The PD mixture has been used for two weeks after preparation. Adequate digestive power is maintained, although a certain loss of the enzyme activity seems to occur. A few experiments indicate that the digestive power of the solution is satisfactory also after storage for one month.

It seems that the digestive power of pancreatin is increased by addition of quaternary ammonium compounds.

Experiments with various quaternary ammonium compounds are being carried out. The compounds vary with respect to their effect on contaminants and on tubercle bacilli. Compounds other than the one here reported might be used with advantage.

The method described is time saving and very simple.

It seems that the risk of laboratory infection would be reduced with this method, because fewer manipulations are required. The neutralization and centrifugation procedures of the NaOH method are eliminated. It might seem practicable to conduct the PD method directly in the universal containers in which the sputum specimens are received. In consequence, the PD method will be carried out in a more closed system than the NaOH method. However, the final evaluation of these aspects of the method will have to wait until it is known whether the sensitivity of the method will be increased by centrifugation.

A more detailed report on the subject will be given elsewhere in due time.[4]

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July 6, 1953

[4] A further experimental series has shown only about equal results with the NaOH and PD methods. This apparent deterioration of the PD results is not statistically significant at the 5 per cent level and may be due to chance, but the possibility should be considered that the method might be unfavorably influenced by unknown variable factor(s). A study of several variable factors is in progress.

POSITIVE M. TUBERCULOSIS CULTURES OBTAINED BY INCUBATION BEYOND THF NORMAL SEVEN- OR EIGHT- WEEK PERIOD

Following the Twelfth Veterans Administration-Army-Navy Conference on the Chemotherapy of Tuberculosis, a revision of the diagnostic laboratory methods was published by the Subcommittee on Laboratory Methods.[1] A statement in Section II of this revision, concerning the incubation time, prompted this analysis to determine the importance of prolonged incubation with reference to routine sputum and gastric specimens. It has been the policy of this laboratory to incubate all cultures for an indefinite period limited only by incubation space.

In an analysis of 10,000 cultures of possibly tuberculous material, 3,457 (34.6 per cent) were reported as positive for *M. tuberculosis*, 5,993 (59.9 per cent) as negative, and 550 (5.5 per cent) lost through contamination and laboratory accidents during the normal seven-week incubation period. All negative cultures were held for continued incubation. Forty-five (0.75 per cent) of this negative group became positive for *M. tuberculosis* during the period ranging from nine to twenty-four weeks of cultural incubation (table 1).

TABLE 1

WEEKS OF INCUBATION.....	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL POSITIVE
Number of cultures positive for *M. tuberculosis*.....	2	1	7	1	3	2	13	0	5	1	3	3	2	1	0	1	—	45

Forty-two patients were represented by the 45 "late-positives." Specimens from 3 patients yielded "late-positives" on two occasions. All had received one or more of the antituberculous drugs for their disease. The materials submitted were 42 specimens of sputum and 3 of gastric washings. Stained preparations for acid-fast bacilli were made on the sputum specimens; 31 were reported as negative, and 11 as positive, for acid-fast bacilli.

No significant trend could be attributed to any specific drug or combination of drugs. However, with one exception, all "late-positive" cultures were from specimens received during the post-therapy period. Drug-susceptibility studies of the isolated organisms were consistent with previously reported susceptibilities. Cultures of *M. tuberculosis* which appeared within the normal seven-week incubation period were previously, and have been subsequently, isolated from specimens collected from these same patients.

It is probable that the extended incubation of routine specimens is of minimal importance for the following reasons.

In no instance was the "late-positive" culture of *M. tuberculosis* of diagnostic value: positive cultures were isolated consistently from the same patients during the normal incubation period from other specimens.

The drug-susceptibility studies were comparable to previously reported susceptibilities from the same patients.

[1] Diagnostic laboratory methods as revised following the Twelfth Veterans Administration-Army-Navy Conference on the Chemotherapy of Tuberculosis, Atlanta, Georgia, February 9-12, 1953.

The method of handling and adequacy of the specimen are more important than the extension of incubation time.

RESEARCH AND DEVELOPMENT BRANCH
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August 20, 1953

Books

SYMPOSIUM ON COAL MINER'S PNEUMOCONIOSIS. Pp. 95, The Golden Clinic, Memorial General Hospital, Elkins, West Virginia, 1952.

The subject matter of this book is both timely and important, not only from the academic, but also from the practical, point of view. The topic is of academic interest chiefly because the condition has been rather recently described and is thought by some to be caused specifically by exposure to coal dust and to be unrelated in its etiology and pathogenesis to silicosis or other forms of pneumoconiosis. The topic is of practical interest because it raises new problems concerning compensation and employment of persons exhibiting evidences of the condition.

The book presents a series of six lectures given by five individuals at a symposium held in November of 1952. The casual reader, wondering what is meant by the term "coal miners' pneumoconiosis," will be able to extract its significance by careful reading of the book. However, not one of the speakers attempts a concise definition. The general lack of concise and strict definitions of various terms used in this field is also apparent in the book since one and the same author is found using the terms "silicosis" and "pneumoconiosis" interchangeably at one point, and as having distinct meanings at another point in the same lecture.

The student of pneumoconiosis may expect to find in this book demonstrations of the reality of a specific condition occurring in coal miners and of its significance. Here the book is lamentably inadequate since none of the many illustrations and tabulations of data presented in the lectures are reproduced in the book. It is thus impossible for the reader to review, analyze, and evaluate objective findings. This reviewer realizes the practical problems of size and expense in publishing books of this type, but believes that key tables and illustrations are essential and should have been included at any cost. It would be desirable also to include references to the literature when previously published communications are quoted.

As presented by one speaker, coal miners' pneumoconiosis develops gradually and passes through at least three stages as follows: first, focal accumulation of dust, especially at the points of branching of terminal bronchioles; second, the development of focal emphysema at such sites of localization. It is noted that fibrosis is inconspicious or absent in these two stages but, in the third and final one, conglomerate fibrous masses occur, usually in the upper lobes, with extensive emphysema elsewhere. It is indicated that these stages can be distinguished roentgenographically. The fibrotic masses are attributed to the presence of infection in addition to the dust, and it is suggested that the infection is probably tuberculous. The occurrence of disability resulting from pulmonary damage is stressed, but it is not clearly indicated whether such disability is of significant degree in the first two stages.

The most startling concept presented in the book is that of Dr. Hugh-Jones of the British West Indies, who suggests that the emphysema in coal workers' pneumoconiosis is different from spontaneous emphysema as it occurs in non-miners. His statement in this connection follows:

> But what is so interesting is that, on the whole, the residual air itself remains relatively *constant* in coal workers' pneumoconiosis. What is happening is that the total lung volume is falling. That is in contrast to the cases of emphysema we got from London, who had a marked rise in residual capacity. They also happened to have a large total lung volume, though the percentage is still markedly raised. Thus, you can get a change in the percentage of residual air for two reasons: either the

total capacity gets smaller or the residual air gets larger. It does seem to me to be confusing to make the two changes synonymous with "emphysema".

This reviewer cannot allow these statements to go unchallenged. Let us assume that a lung has a certain total volume and a certain residual air. Let us now consolidate one lobe of that lung, for example, by filling it with paraffin. This procedure would simulate in certain respects the massive fibrotic stage of coal miners' pneumoconiosis. It is obvious that the total lung volume will be decreased by the volume now occupied with paraffin. It should also be clear that the residual air will be similarly decreased by the volume of that air which previously would have been present in the now consolidated tissue. Since, in the coal worker, the residual air does not decrease but remains constant as the massive fibrosis consolidates the upper lobes, it appears to this observer that the trend toward increasing residual air is present in the emphysema associated with coal workers' pneumoconiosis even as it is in spontaneous emphysema. It seems, therefore, that in conditions of this type attention should be paid to the relationship between residual air and total lung volume rather than to the absolute quantity of residual air.

In summary, it may be said that the rather casual reader seeking a general conception of coal miners' pneumoconiosis should find this book useful. The student of pneumoconiosis, seeking evidence concerning the etiology, pathogenesis, and clinical significance (disability) of coal workers' pneumoconiosis, will be disappointed.

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ASPECTS OF THE PSYCHOLOGY OF THE TUBERCULOUS. By Gordon F. Derner. Pp. 119, Paul B. Hoeber, Inc., New York, 1953, Price $3.50.

This short monograph describes in nine chapters and 117 pages the efforts of the author to determine whether or not there is something special about the behavior of people who have tuberculosis. He is also attempting to learn more about their reactions to their illness and to hospitalization.

The method used consists of subjecting the patients to a series of standard psychometric tests and additional tests especially designed for this study. Most of the chapters of the book are devoted to the details and results of the tests. The author was unable to find any common denominator in the patients with tuberculosis other than the disease itself, and no definite statement can be made which would lead one to suspect that there is something special about the personality structure of patients with tuberculosis. Throughout the work no serious attempt was made to correlate periods of stress in the patient's life with the onset of the illness, although some of the patients seemed to be aware of this correlation. The work points up the futility of pursuing this problem and allied problems with the method of multiple psychometric tests as in common use today. This method has not been successful in the past and, indeed, there is little reason to believe it will continue to be any more successful than it has been.

Perhaps one of the reasons for the failure of this technique of facing the patient with multiple psychometric tests is that the person who is conducting the test has (*1*) failed to ask himself any questions, and (*2*) has failed to understand the holistic point of view—that is, the individual is reacting to a situation and that his adaptation is in some way linked with his resistance to tuberculosis. It is somewhat surprising that the author has failed to indicate the notions of René Dubos on this subject.

WILLIAM J. GRACE
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THE METABOLISM OF THE TUBERCLE BACILLUS. By William F. Drea and Anatole Andrejew, with a foreword by Esmond R. Long. Pp. 448, Charles C Thomas, Springfield, Illinois, 1953, Price, $12.50.

Though recognizing full well the difficulties inherent in writing a book dealing solely with the metabolism of the tubercle bacillus, the reviewer must express disappointment in the present volume.

It is a reasonably safe assumption that, if one were asked to list the most significant recent developments concerned with the metabolism of the tubercle bacillus, the effect of *p*-aminosalicylic acid on the growth and metabolism of the organism would be high on the list. It is, therefore, surprising that, in *The Metabolism of the Tubercle Bacillus*, one looks in vain for any mention of this compound. Unfortunately, this is not the only such omission. A great deal of emphasis is placed upon experiments performed between 1920 and 1938. These data are reported uncritically, and no attempt is made to evaluate them in terms of more modern techniques. For example, earlier work dealing with mineral requirements almost certainly needs revision in light of present-day knowledge of trace metal requirements. It is difficult to believe that phosphorous, sulfur, magnesium, and potassium satisfy the essential mineral requirements of the organism.

The introductory pages of the chapter on enzymes appear out of place in a volume such as this. This loose consideration seems to obfuscate rather than clarify. Similarly, the derivation of formulas for determining Warburg flask constants would seem to be outside the purview of the present volume, and rather left to standard volumes on technique.

One wonders also about the value of discussing in considerable detail the beautiful, but frequently reviewed, methodology and results of R. J. Anderson and associates. Would not a simple summary of results and discussion of the implications here have been sufficient?

However, the chief weakness of the present volume is the superficiality with which the authors discuss the metabolism of the organism. The pages dealing with the respiratory and enzymatic activity of the mycobacteria are merely an uncritical compilation of published data, with no attempt at interpretation or evaluation in terms of comparative biochemistry.

In the reviewer's opinion, another serious fault of the volume lies in the fact that the authors desired to "... prepare a useful book for the greatest possible number of investigators in tuberculosis (physicians, bacteriologists, immunologists, histologists, and so forth) and not solely for some specialized biochemists ..." (authors' preface). One can sympathize with this aim, indeed devoutly hope for its consummation; but such an attempt may, as in the present volume, eventuate in a book of only limited value to workers in any one of these areas.

It should be pointed out that there are solid sections in the book. The imaginative work of Drea and associates on depth growth of tubercle bacilli is beautifully described. Some sections on tuberculoprotein are interestingly and informatively written.

As one reads the volume, one becomes increasingly aware of the tremendous gaps in our knowledge of the intermediate and intimate metabolism of the tubercle bacillus. Perhaps the time has not yet come for a volume of this sort; perhaps not until the "specialized biochemists" investigate in greater detail the metabolism of the wonderfully complex puzzles we call mycobacteria can we look for a book on metabolism useful "for the greatest number of investigators in tuberculosis."

ARTHUR K. SAZ
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BOOKS RECEIVED

CHLOROPHYLL IN MEDICINE. Pp. 53, Rystan Company, Inc., Mount Vernon, New York, 1953.

RESPIRATORY DISEASES AND ALLERGY. By Josef S. Smul. Pp. 80, Medical Library Company, New York, 1953, Price, $2.75.

LIVING WITH A DISABILITY. By Howard A. Rusk and Eugene J. Taylor, in collaboration with Muriel Zimmerman and Julia Judson. Pp. 207, The Blakiston Company, Inc., Garden City, New York, 1953, Price, $3.50.

TUBERCULIN PATCH TEST SURVEY AMONG KINDERGARTEN AND FIRST-GRADE CHILDREN IN PUBLIC ELEMENTARY SCHOOLS, QUEENS COUNTY, NEW YORK. Pp. 6, Queensboro Tuberculosis and Health Association, 159-29 90th Avenue, Jamaica 2, New York.

TREPHINE TECHNIQUE OF BONE MARROW INFUSIONS AND TISSUE BIOPSIES. Sixth Edition. By Henry Turkel. Pp. 78, Trephine Instruments, Inc., Detroit, Michigan, 1952.

American Trudeau Society

Medical Section of the National Tuberculosis Association

Report of the Committee on Therapy to the Committee on Medical Research, October 14–15, 1953

The Committee on Therapy of the American Trudeau Society met at the Hotel New Yorker, New York, N. Y., on October 12 and 13, 1953. All members were present, viz., Dr. Robert H. Ebert, chairman, Dr. Paul Chapman, Dr. Raymond F. Corpe, Dr. Robert A. Goodwin, Dr. Roger S. Mitchell, Dr. Carl Muschenheim, Dr. Edward F. Parker, Dr. Morris C. Thomas, and Dr. Robert L. Yeager. Since the composition of the Committee was largely new, a major portion of the meeting was devoted to consideration of new topics for study.

1. *Preparation of a Manual on Pembine-type Conferences*

The success of the Pembine Conference has attracted much attention, and conferences of the Pembine type have been inaugurated in many parts of the country. Some groups have been interested in starting such conferences, but have been uncertain as to the methods and procedures that shculd be used. To help such groups, the Committee on Therapy has undertaken the preparation of a guide for the planning of a Pembine-type Conference. Dr. Morris Thomas presented a preliminary draft of a manual for consideration by the Committee, and members were asked to send him comments and criticisms before November 15, 1953. It was the consensus that the manual should be mimeographed and made available through the central office of the American Trudeau Society to those interested. It was suggested that after completion an announcement could be made in the American Trudeau Society News Letter that such a manual had been prepared.

2. *Tuberculous Meningitis*

Tuberculous meningitis remains a formidable disease in spite of advance in therapy. Last year's Committee thought that a statement should be written offering a guide to the physician who treats an occasional case of meningitis. A preliminary statement prepared by last year's Committee was reviewed, and Dr. Chapman was asked to revise the statement with instructions to give a more critical review of all aspects of the problem.

3. *Tuberculous Lymphadenitis*

Tuberculous lymphadenitis is seen often enough to present a problem in therapy; yet there seems to be little agreement as to the best methods of treatment. It was decided to pool the knowledge of the Committee, and each member was asked to prepare a summary of his recent experiences in the treatment of this form of tuberculosis for presentation at the next meeting. Dr. Muschenheim consented to review the recent literature on the subject.

4. *Tuberculosis of the Eye*

There appears to be similar confusion concerning treatment of tuberculosis of the eye. Dr. Mitchell offered to review the literature on the subject and evaluate the problem for the Committee.

5. *Bed Rest and Ambulatory Chemotherapy*

Some of the difficulties inherent in the problem of evaluating the role of bed rest in the treatment of tuberculosis were discussed. It was thought that the Committee should prepare a statement on the subject of bed rest in patients receiving chemotherapy even though the data are incomplete. Dr. Yeager consented to prepare such a statement.

6. *Surgery*

The pros and cons of resection of residual pulmonary lesions were discussed, and it appeared that no definite decision could be made about the merits of resection until more time had elapsed and more was known about the bacteriology of the acid-fast bacilli present in these necrotic lesions after prolonged chemotherapy. Dr. Corpe was asked to prepare a statement which would outline the dilemma and which would also re-emphasize the valuable role of surgical collapse in the treatment of pulmonary tuberculosis.

7. *A Filmstrip on Chemotherapy*

The Committee believed that there is need for a filmstrip on chemotherapy for the education of tuberculosis patients and recommends that such a film be made. Such a film should particularly emphasize the need for continued long-term chemotherapy and the dangers of interrupted treatment.

Dr. Mitchell suggested this topic and told the Committee that Dr. Arthur Hellwig, who makes Hellwig PAS products, might be interested in paying for such a filmstrip made under the auspices of the American Trudeau Society.

8. *Tuberculosis in Children*

Correspondence from Dr. Walcher was read concerning the lack of interest of pediatric societies in tuberculosis. Dr. Walcher asked in his letter for "comments on the advisability of attempting to initiate a movement through one of the pediatric organizations, thereby integrating and coordinating some of our efforts and thoughts on the entire problem of tuberculosis in children."

The Committee thought that Dr. Walcher's plan should be given every encouragement and suggested names of persons who might be interested in promoting such a program. The Committee on Therapy also believed that the Program Committee might encourage interest on the part of pediatricians by judicious planning of the annual program. Similarly, the suggestion was made that more emphasis should be placed on extrapulmonary tuberculosis in future programs so that the interest of orthopedists, urologists, and other specialists who treat tuberculosis might be stimulated.

9. *Meeting with Laboratory Subcommittee*

A joint meeting was held with the Laboratory Subcommittee in the evening of October 12. The problem of avirulence of isoniazid-resistant bacilli was discussed and material was presented which indicated that avirulence for one host (e.g., the guinea pig) did not necessarily mean avirulence for another host (e.g., the mouse). Evidence was presented that patients from whom isoniazid-resistant tubercle bacilli can be recovered may relapse or die while on therapy. This does not mean that isoniazid therapy should be stopped if resistant organisms are recovered from the sputum. But it does indicate that isoniazid-resistant organisms can be virulent for the human host, even though avirulent for the guinea pig.

The Committee on Therapy adjourned at noon October 13 and plans to hold its next

meeting in St. Louis on February 6 or 7, 1954, just before the Thirteenth Veterans Administration-Army-Navy Chemotherapy Conference.

Respectfully submitted,

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RAYMOND F. CORPE, M.D.
ROBERT A. GOODWIN, JR.
ROGER S. MITCHELL
CARL MUSCHENHEIM
EDWARD F. PARKER, M.D.
MORRIS C. THOMAS, M.D.
ROBERT L. YEAGER, M.D.

ROBERT H. EBERT, M.D., *Chairman*
Committee on Therapy

Report of the Laboratory Subcommittee to the Committee on Medical Research, October 14–15, 1953

The Laboratory Subcommittee of the Committee on Medical Research met at the Hotel New Yorker, New York, N. Y., on October 12, 1953, beginning at 3:00 p.m. A joint session was held in the evening with the Committee on Therapy. Dr. Ebert, chairman, will report on the deliberations at the joint session.

All members were present for the meetings. The following is a report of the discussion:

1. The Subcommittee wishes to reaffirm a statement previously made, viz., that most of the mutants of tubercle bacilli which are highly resistant to isoniazid *in vitro* are nonpathogenic for guinea pigs under certain experimental conditions, but that, on the other hand, there is no clinical evidence that such strains are nonpathogenic for human beings.

2. Some isoniazid-resistant mutants of tubercle bacilli are highly pathogenic for guinea pigs.

3. The Subcommittee urges that more extensive studies be made of the pathogenicity of different isoniazid-resistant mutants of tubercle bacilli in different species of experimental animals.

4. Some isoniazid-resistant mutants of these organisms, although proved nonpathogenic for guinea pigs, have been found, nevertheless, to possess significant pathogenicity for at least one strain of mouse.

5. The Subcommittee would point out that inadequate data are available concerning the possible loss of virulence of tubercle bacilli when they become resistant to other individual chemotherapeutic agents or combinations thereof, or concerning the spontaneous loss of virulence of these organisms in untreated patients.

6. Studies also are needed on strains of mycobacteria which are associated with granulomatous disease in human beings but which are avirulent for the guinea pig and are in other respects not bacteriologically classifiable as tubercle bacilli.

Respectfully submitted,

Myron W. Fisher
Meyer R. Lichtenstein
Charles E. Lyght
Kornel L. Terplan
Emanuel Wolinsky
Diran Yegian

Gardner Middlebrook, M.D., *Chairman*
Laboratory Subcommittee

NOTES

POSTGRADUATE COURSE IN THE MEASUREMENT OF PULMONARY FUNCTION

A postgraduate course in the measurement of pulmonary function in health and disease will be held for physicians in the New England area at Boston City Hospital, March 22–26, 1954, under the sponsorship of the American Trudeau Society, in cooperation with the Massachusetts Tuberculosis and Health League, Harvard Medical School, Boston University School of Medicine, and Tufts College Medical School.

The course is being held for the purpose of presenting the fundamental concepts and methods of analysis of normal and abnormal physiology of the lungs in a manner which can be readily understood by physicians unfamiliar with this type of chest work. Applications and more detailed information may be obtained from Edward J. Welch, M.D., chairman, ATS Regional Committee on Postgraduate Courses, 1101 Beacon Street, Brookline 46, Mass.

ANNOUNCEMENT OF THE TRUDEAU SCHOOL OF TUBERCULOSIS FOR 1954

Fortieth Annual Session

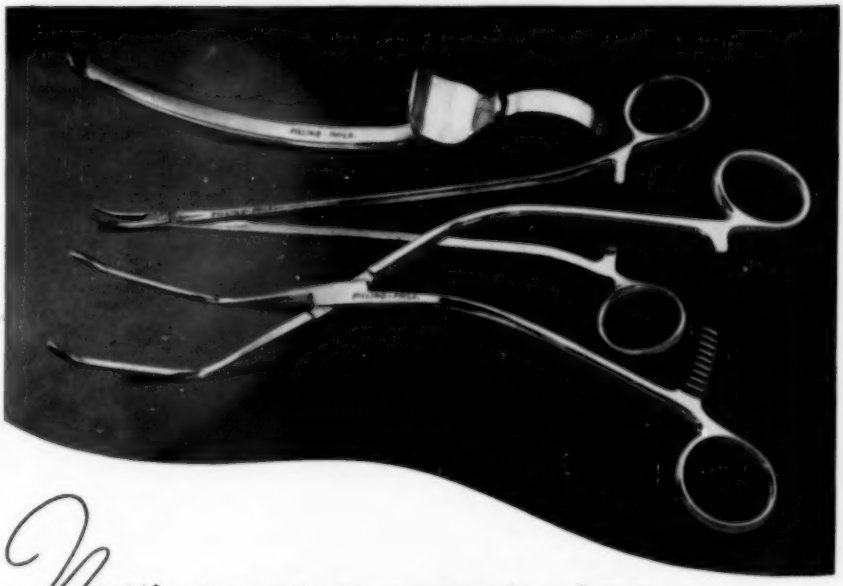
The Trudeau School of Tuberculosis will present its fortieth annual session, beginning Tuesday, June 1 and concluding June 25. The course will cover all aspects of pulmonary tuberculosis and also certain phases of other chronic chest diseases including those of occupational origin.

The schedule for the 1954 course is in preparation, but copies of the program for 1953 are available.

Inasmuch as registration is limited and reservations have already been requested for the 1954 session, it is suggested that those who plan to attend make early application for enrollment.

The tuition fee is $100, payable to the Trudeau School on or before the opening date, June 1, 1954. A few scholarships are available for those individuals who can qualify. The Trudeau School of Tuberculosis has been approved for training of Veterans under Public Laws, and those desiring to obtain Veterans' benefits should clear their registration with the Veterans Administration before the session begins.

Communications should be addressed to: Secretary, Trudeau School, Saranac Lake, New York.



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Research Facilities Available: Saranac Laboratory; Trudeau Laboratory; Physiology and Biochemistry Laboratories; Ray Brook State Hospital Laboratory, and the X-Ray Laboratories of Trudeau, Ray Brook and Stony Wold Sanatoriums.

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Note: Since 1939 the Trudeau School has been supported by grants from the Lillia Babbitt Hyde Foundation, of which Mr. Charles C. Harris is President.

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1. Hobby, G. L., Lenert, T. F., Rivoire, Z. C., Donikian, M., and Pikula, D.: Am. Rev. Tuberc. 67:808, 1953.

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on TUBERCULOSIS and ALLIED DISEASES

●**FUNDAMENTALS OF PULMONARY TUBERCULOSIS AND ITS COMPLICATIONS.** American College of Chest Physicians *edited by* E. W. Hayes, M.D., Coll. of Med. Evangelists. "A successful presentation of the chief aspects of tuberculosis treatment and control, with emphasis on modern developments."—*Radiology*. 1949.

492 pp . 219 illus . $9.50

●**THE METABOLISM OF THE TUBERCLE BACILLUS.** *By* William F. Drea, D.M.D., Colorado Foundation for Research in Tuberculosis, and Anatole Andrejew, I.C., Pasteur Institute, Paris. In bringing out this practical application of new facts on the bacillus in diagnosis and chemotherapy, the authors have formed a highly useful service for investigators.

1953 . 470 pp . 24 illus . $12.50

●**TUBERCULOSIS AMONG CHILDREN AND ADULTS** (3rd Ed.). *By* J. Arthur Myers, M.D., Univ. of Minnesota. "This book is most complete in its scope, and yet it is written in a concise style which has enabled the author to include all of the pertinent facts of present-day thinking on this important subject in a single volume."—*J. Pediat.*

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●**THE CHEST AND THE HEART.** *By* J. Arthur Myers, M.D., *and* C. A. McKinlay, M.D., both of Univ. of Minnesota. "It is the first time the chest as a whole has been dealt with in one treatise . . . the various chapters are so well integrated in subject matter that it is one of the best textbooks on the subjects discussed . . . written by the best in the field."—*J.A.M.A.*

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●**THE PATHOGENESIS OF TUBERCULOSIS** (2nd Ed.). *By* Arnold R. Rich, M.D., The Johns Hopkins Univ. "It is a critical perspective of the entire picture of the pathogenesis of tuberculosis, wide in scope, penetrating in analysis, stimulating, provocative, exhaustive in facts and conservative in conclusions."—*Arch. of Path.*

1952 . 1056 pp . 89 figs . $15.00

●**DIAGNOSTIC AND EXPERIMENTAL METHODS IN TUBERCULOSIS** (2nd Ed.). *By* Henry S. Willis, M.D., North Carolina Sanatoria, *and* Martin M. Cummings, M.D., Lawson V.A. Hosp. "This book should have an enthusiastic reception by students, general practitioners, pulmonary disease specialists and laboratory workers alike, for nowhere else is there such a clear and practical presentation of the fundamental laboratory aspects of tuberculosis."—*Am. J. Med. Sc.*

1952 . 408 pp . 41 illus . $10.00

●**THIS IS YOUR WORLD.** *By* Harry A. Wilmer, M.D., San Mateo County Tuberculosis Sanatorium, San Francisco. "This wonderful little book is simply a must for any psychiatrist interested in rehabilitation. Not a coldly scientific book, it is really an emotional experience."—*Am. J. Psychiat.*

●**LUNG ABSCESS.** *By* R. C. Brock. Describes basic surgical anatomy and pathology of lung suppuration and continues with an analysis and description of the various forms of lung abscess.

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●**RADIOLOGIC EXPLORATION OF THE BRONCHUS.** *By* S. Di Rienzo, M.D., Univ. Cordoba, Argentina. "Occasionally one is privileged to review a medical volume which is a work of art in addition to being a book of factual medical material. The author has succeeded admirably in his purpose of demonstrating abnormalities in bronchial anatomy and physiology by radiographic means."—*Dis. of Chest*

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●**CYTOLOGIC DIAGNOSIS OF LUNG CANCER.** *By* Seymour M. Farber *et al.*, Univ. Calif. "The authors have succeeded in furnishing guidance to cytologists and have surpassed earlier investigators in presenting sensible and practical solutions to the many problems of application of the cytological method."—*Arch. Path.*

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●**TREATMENT OF RESPIRATORY EMERGENCIES INCLUDING BULBAR POLIOMYELITIS.** *By* Thomas C. Galloway, M.D., Northwestern Univ. "Respiratory emergencies are considered from the point of view of a) The Mechanics of Respiratory Obstruction, b) Anoxia in Clinical States. The conclusions drawn from this double approach are brought to a focus in the specific problem of Poliomyelitis."—*Quart. Rev. Opth. & Oto.*

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50th ANNIVERSARY ★ NATIONAL TUBERCULOSIS ASSOCIATION
Annual Meeting, Atlantic City, New Jersey, May 17-21, 1954

The 50th Anniversary of the National Tuberculosis Association will be celebrated at its Annual Meeting to be held in Atlantic City, New Jersey, May 17-21, 1954.

Three adjacent hotels, the Ambassador, Chelsea and Ritz-Carlton, will serve as headquarters. Sessions and conferences will be divided among the three hotels with no one hotel marked exclusively for medical or community service programs.

Applications for rooms should be made directly to the hotel of choice. In the event that the hotel is unable to provide the desired space, requests will be turned over to the Convention Bureau, which will clear requests with another one of the cooperating hotels.

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